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Effect on prolactin secretion of *Echinacea purpurea*, *Hypericum perforatum* and *Eleutherococcus senticosus*

G. Di Carlo, M. Pacilio, R. Capasso, R. Di Carlo*

Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy

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Dedicated to the memory of Giulia Di Carlo

Abstract

It has been recently reported that prolactin (PRL) plays an important role in immune system regulation.

In this study we investigated the activity of three natural drugs with immunomodulatory activity: *Echinacea purpurea* (EP), *Hypericum perforatum* (HP) and *Eleutherococcus senticosus* (ES) on PRL production.

Male rats were orally treated with two different doses (30 and 100 mg/kg) of extract of these drugs for 3 or 15 days. A 3-day treatment was not able to modify PRL serum levels, whereas a 15-day treatment with EP and HP at the higher dose significantly inhibits PRL production.

A treatment with ES was always ineffective. A possible mechanism for this effect could be that both HP and EP extracts display a direct dopaminergic activity, although an involvement of the GABA-ergic system cannot be excluded.

Keywords: *Echinacea purpurea*; *Hypericum perforatum*; *Eleuterococcus senticosus*; Prolactin serum levels

Introduction

Prolactin (PRL) is a pituitary hormone to which over 80 different actions have been ascribed (Nicoll, 1974). In fact, its activities are not only related to milk production by mammary glands in animals and humans (activity by which takes its name), but range from pro-inflammatory to modulation of salt and water transport. Recently, it has been reported that PRL also plays an important role in immune system regulation (Murphy et al., 1995; Ben-Jonathan et al., 1996; Weigent, 1996).

Echinacea purpurea (EP), *Hypericum perforatum* (HP) and *Eleutherococcus senticosus* (ES) are natural drugs with immunomodulatory activity. In particular EP has been reported to increase the defence against various kinds of infections (Steinmuller et al., 1993; Barrett, 2003), particularly in patients with defective cellular immunity (See et al., 1997) and it is used in phytotherapy for the treatment of first aerial and urinary ways infections (Bauer, 1998; Percival, 2000; Schulten et al., 2001). Also HP has been largely used by folk and phytotherapy medicine for its good antiinflammatory, antiseptic and antidepressant activity, and it has also been proposed to be useful as antiviral agent (Barnes et al., 2001; Di Carlo et al., 2001; Kumar et al., 2001). Finally, ES, better known as the Siberian ginseng, that is

*Corresponding author. Tel.: +39 081 678413; fax: +39 081 678403.
E-mail address: dicarlo@unina.it (R. Di Carlo).

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an adaptogen (i.e. able to increase non specific body resistance to stress and fatigue), has been reported to enhance body natural defences against various pathogen agents (Steinmann et al., 2001; Szolomicki et al., 2000).

While a hypoprolactinemic activity of HP was recently described (Franklin et al., 1999; Winterhoff et al., 1995), no data are available in literature on the effect of the other two drugs on PRL production. So since PRL and EP, HP and ES affect immune system, the aim of our study was to verify if a correlation could exist between a treatment with EP, HP, and ES and PRL production.

Materials and methods

Plant material

EP root dry powder, HP flowering top dried extracts and ES root dry powder were kindly provided by Arkopharma (Carros, France). EP contained 1.5% of total polyphenols, calculated as chlorogenic acid. HP contained 0.27% of anthraquinone derivatives, calculated as hypericin, and 2.5% of hyperforin; the extract was not characterized for flavonoids. ES contained 0.4% of eleutherosides. The drugs were powdered and suspended in carboxymethylcellulose 1%.

Chemicals

Carboxymethylcellulose and ketamine were obtained from Sigma (Milan, Italy), fetal bovine serum, horse serum, culture media and supplements were purchased from Hy-Clone (Road Logan, UT, USA).

Animals and treatment

Male Wistar rats (Harlan, Italy) weighing 150–160 g were used after a week acclimatization in housing conditions (temperature 23 ± 2 °C, humidity 60%) with a fixed 12 h light-dark cycle and received food and water ad libitum for the whole period of experimental manipulations. All animal experiments complied with the Italian D.L. no. 116 (27 January 1992) and associated guidelines in the European Communities Council Directive (24 November 1986). Animals (8 rats per group) were treated intragastrically with EP, HP or ES (30 and 100 mg/kg) suspended in carboxymethylcellulose 1% (CMC) (0.5 ml/100 g) for 3 or 15 days. The control group received the same volume of vehicle (CMC). Two hours after the last treatment the animals were anaesthetized with ketamine (200 mg/kg) and killed by bleeding. The blood was then stored overnight in glass vials at room temperature for the Nb2 assay.

Determination of prolactin serum levels by biological assay

It is well known that multiple molecular forms of immunoreactive PRL exist with differences in molecular weight and biological activity. This heterogeneity with differing biological activity may explain in part why radioimmunoassay measurements do not always correlate with clinical findings (Maddox et al., 1989; Peabody et al., 1992). On the other hand it has been suggested that anti-PRL antibodies give variable results on serum PRL measurements depending on the immunoassay used (Hattori et al., 1994).

An indirect method to assess serum PRL levels that avoids these inconveniences is the determination of PRL bioactivity with a biological assay, the Nb2 lymphoma cell proliferation.

In our study we used rat node lymphoma estrogenized cells of Noble strain (Nb2) kindly given by Doctor Vincent Goffin of the INSERM-U44 of Paris. This is a particular kind of cells whose growth is stimulated by PRL (Maddox et al., 1989) and so PRL concentration in a biological fluid may be extrapolated comparing their growth stimulated by the hormone in respect to a standard PRL concentration curve plotted against optical density.

Nb2 lymphoma cells were cultured in RPMI 1640 medium containing 10% horse serum, 10% inactive fetal calf serum (56 °C for 30 min), 50 mM β-mercaptoethanol, 20 mM Hepes, 500 U/ml penicillin and 500 μg/ml streptomycin. The biological assay for PRL serum determination was performed as previously described (Pacilio et al., 2001).

As standard rat PRL (RP-3), a gift from the National Institute of Diabetes and Kidney Disease (NIDDK), was used. Standard curve for PRL concentration (from 30 pg to 10^4 pg/ml) was plotted against optical density; the hormone concentration being expressed in $\log_{10}$ pg/ml PRL. Unknown concentrations of PRL in the serum samples were calculated by measuring the hormone concentrations off the standard curve. The intrassay and interassay coefficients of variation were 1.4–2.7% and 3.9–6.5%, respectively.

Statistical analysis

All data are expressed as mean ± SE. Statistical evaluation of results was done using Dunnett's test. $P < 0.05$ was considered significant.

Results and discussion

A treatment with EP, HP or ES for 3 days was not able to significantly modify PRL concentrations in

tested sera (data not shown). On the contrary, a more prolonged treatment (15 days) with EP, HP at the higher dose (100 mg/kg) significantly ($P<0.05$ and $P<0.01$, respectively) inhibited PRL production in treated animals, while a treatment with ES was ineffective (Fig. 1). No signs of abnormal behaviour were observed in the animals during the treatment period.

The findings suggest that both EP and HP could have an inhibitory activity on PRL production after repeated administrations. As it is well known that dopamine suppress PRL secretion (Tuomisto and Mannisto, 1985), a possible explanation for these results could be that both HP and EP enhance some steps of dopamine neurotransmission, increasing dopamine availability, or act directly at pituitary level with a dopamine-like mechanism. In particular, as reported by Muller et al. (1997), HP does have the ability to block dopamine re-uptake more potently than 5-HT or NA in rodents. Moreover, a possible involvement of GABA should also be considered. Actually, as reported by Chatterjee et al. (1998), HP has a clear inhibitory effect on the neuronal uptake of GABA, a transmitter involved in the regulation of PRL release (Leong et al., 1983). On the contrary no data are available concerning EP activity on the dopaminergic or GABA-ergic system. Anyway it is interesting to notice that chlorogenic acid, one of the main Echinacea components, presents some structural similarities with dopamine.

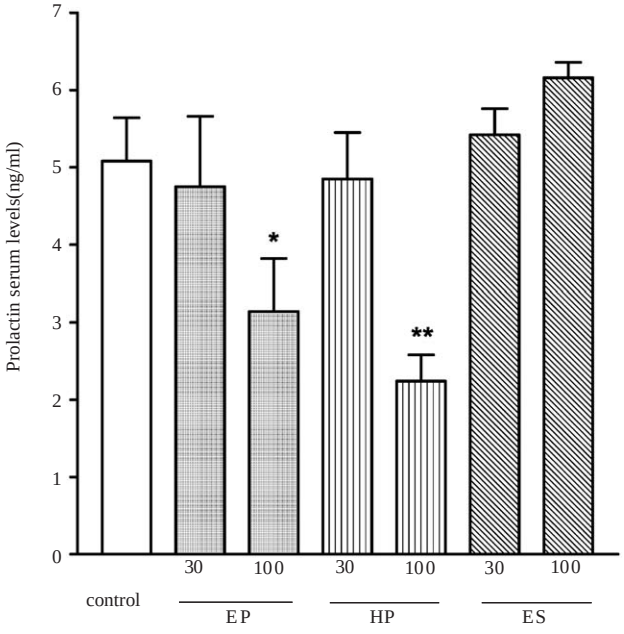

Fig. 1. Prolactin serum levels (mg/ml ± SE) in male rats treated intragastrically with *Echinacea purpurea* (EP), *Hypericum perforatum* (HP) or *Eleutherococcus senticosus* (ES), 30 or 100 mg/kg/die for 15 days. The data are means ± S.E. of 8 animals. $^{*}P<0.05$ vs. control, $^{**}P<0.01$ vs. control.

Concerning HP activity our data are in agreement with the results reported by Franklin et al. (1999, 2000) both in animal and human studies, whereas Butterweck et al. (2001) did not found an effect of repeated treatment with HP on rat plasma PRL levels.

However, in a previous paper the same authors reported that an extract of St. John's wort significantly decreased plasma PRL levels after repeated treatment (Winterhoff et al., 1995). A possible explanation for the different results observed could be found in the different composition of the various HP extracts used and in the different doses administered. For instance, in the study of Butterweck et al. (2001), a higher dose (500 mg/kg) of an extract enriched with hyperforin (5%) was used.

No data are reported at present about the hypoprolactinemic activity of EP. This new finding should be taken into account, considering that preparations from EP are among the most widely used herbal medicine.

As regards the possible clinical implication of our findings we remind that elevated PRL secretion can lead in the non-pregnant woman and in males to a series of clinical and biochemical effects as a result of central and peripheral action of the hormone: mainly subfertility and galactorrhea in both sexes, failure to enter menarche and amenorrhea in the female, signs of androgen failure in the male. On the contrary a condition of hypoprolactinemia has not yet been described with the exception of postpartum, but luteal inadequacy was observed following pharmacologically induced low PRL secretion, probably as result of hormone oversuppression (Bohnet et al., 1977; Schulz et al., 1978).

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