Original paper

CIMICIFUGA RACEMOSA EXTRACT FOR THE TREATMENT OF CLIMACTERIC COMPLAINTS

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SUMMARY

Cimicifuga racemosa (CR) is widely used to treat climacteric complaints. These complaints, particularly hot flushes, are associated with over-activity of GnRH pulse generator in the hypothalamus, which is significantly reduced by E_2. These effects can be ideally studied in ovariectomized (ovx) rats. In the present contribution we show that over-activity of GnRH pulse generator in the rat model is reduced by E_2 and by CR BNO 1055 as reflected in the reduced LH pulse amplitudes. This effect is not mediated by the pituitary because the extract had no effect on LH and TSH secretion but significantly inhibited prolactin release from cultured pituitary cells indicating the presence of dopaminergic compounds. Both, E_2 and CR treatment of ovx rats had profound effects on the expression of ER-alpha and ER-beta genes in the medio-basal hypothalamus and the preoptic area and both the compounds stimulated tyrosine hydroxylase gene expression in the preoptic area. This hypothalamic site is the location of GnRH perikarya. It can, therefore, be concluded that catecholaminergic mechanisms are involved in the modality by which the E_2 and CR inhibit the over-activity of the GnRH pulse generator in ovx rats and in postmenopausal women.

Keywords: *Cimicifuga racemosa*, climacteric complaints, estradiol-17ß, hypothalamus

INTRODUCTION

Phyto-estrogens are substances with estrogenic activity, produced by plants, due to their structural similarity to estradiol-17-beta (E_2), the most important ligand for estrogen receptors (ER) (1). Phyto-estrogens are increasingly used as replacement for classical hormone therapy of climacteric and postmenopausal women. They resemble estradiol-17-beta (E_2) structurally and, therefore, bind to both ER-alpha and ER-beta whereby they increase transcriptional activity of estrogen responsive cells. In addition, phytoestrogens have antioxidant activities. Here in we briefly review and add some new results concerning the effects and mechanisms of action of *Cimicifuga racemosa*, which is widely used for the treatment of climacteric complaints.

Japanese women have less mammary cancer than Mid-European or Caucasian-Americans. When Japanese women migrate to the USA, the incidence of mammary cancer in the first daughter generation is as high as in the Caucasian-American population, indicating that environmental rather than racial differences are responsible for this phenomenon. The most radical change in lifestyle of the Asian population after migration into the North American sphere is the composition of the food consumed. In Japan the protein needs are primarily covered by soy products where as in the USA, by meat. Therefore, scientists looked for compounds in soy, which may have beneficial effects in the mammary gland. It became soon clear that isoflavones, particularly genistein and daidzein, are estrogenic compounds present in soy, with effects in the mammary gland and it was speculated that they might be responsible for the "Japanese phenomenon" (2, 3). This boosted the sale of soy products and pure isoflavones are sold without medical advice as food additives, and women suffering from climacteric complaint were targeted as the primary clients. Since women are worried about post-menopausal estrogen deficiency-related diseases such as osteoporosis and arteriosclerosis, they were addressed by the companies producing soy preparations It is now quiet clear that the isoflavones have little, if any, effect on climacteric complaints (4, 5). The effects in the treatment of climacteric complaints appear not to be better than the effects of placebo preparations. They may have however, mild anti-osteoporotic effect as shown primarily in animal experiments and some clinical studies (6, 7). The question, however, remains open whether they are putatively harmful to the mammary gland and endometrium. It is now accepted that progestin-unopposed estrogens stimulate the endometrium such that endometrial hyperplasia, and later endometrial cancer, may develop. Indeed, Unfer *et al.* (8) showed, recently, that 3.37 % of women ingesting 150 mg isoflavones developed endometrium hyperplasia where as no endometrial hyperplasia appeared in the placebo-treated

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women. The safety of the mammary gland is another aspect which deserves consideration since there is a debate whether isoflavones may stimulate pre-existing microcarcinomas of the mammary gland (9). There is increasing evidence that the Japanese phenomenon can be easily explained by the fact that the developing mammary gland, i.e., the mammary gland of pubertal girls, reaches a higher degree of differentiation under the influence of isoflavones (10). Highly differentiated tissue tends to develop less cancer. Indeed, recent studies on Japanese girls migrating post-pubertally from Japan to the United States tend to support this notion. Isoflavones may, however, protect the skeletal system of postmenopausal women.

Another plant extract which is increasingly used for the treatment of climacteric complaints stems from the rhizome of *Cimicifuga racemosa* (CR) (11). Since CR extracts do not possess estrogenic activity, they do not bind to ER-alpha or ER-beta. Its proven clinical efficacy in reducing climacteric complaints is of high scientific interest. It appears that CR extracts contain serotoninergic and dopaminergic compounds (12, 13), which may explain the efficacy in ameliorating psycho-somatic climacteric complaints such as hot-flushes, tachycardiac attacks and emotional instability. It is assumed that hot flushes are the result of an over-active GnRH pulse generator, which causes pulsatile GnRH release from the hypothalamus into the portal vessel which links the median eminence with the anterior pituitary. This pulsatile GnRH release is an essential signal for the pituitary gonadotrophs to release LH and FSH in an orderly fashion. In the ovx rat or in the postmenopausal womene low E_2 level causes over-activity of the GnRH pulse generator which is driven by a number of over-secreted neurotransmitters. One or several of these neurotransmitters appear to spill over to temperature- and heart activity-regulating neurons thereby causing hot flushes and the associated galloping heart attacks. Therefore, estrogenic and neurotransmitter-simulating substances may dampen the over-activity of the GnRH pulse generator and, thereby, circumvent the climacteric complaints. Hence, substances that inhibit pulsatile pituitary LH release in ovx rats may be utilized for the treatment of these complaints. Finding if the special CR extract BNO 1055 has such activity has been the objective of the present study.

MATERIALS AND METHODS

We studied the effects of the special CR BNO 1055 in pituitary cells kept under culture conditions and in animal experiments. Details of culture conditions have been published earlier (13). In the supernatants of the cell cultures the concentrations of prolactin, LH and TSH were determined. This allowed conclusions about direct effects of CR BNO 1055 in the pituitary on the release of these hormones. Rats were kept under standardized conditions (soy-free, potato protein-containing food and tap water *ad libitum*). Ovx rats (average weight 330g) were treated with the CR BNO 1055 extract and the acute and chronic effects of this extract in a number of organs, including the brain, were studied. The preparation and characterization of this ethanolic extract has been described previously (14). The treated animals received a single *intra-venous (iv)* injection of 62.5mg CR BNO 1055 dissolved in 1ml of 5% cremophor through an indwelling jugular vein catheter. Positive control ovx rats received 2.5mg E_2 also dissolved in cremophor. The negative control rats received 1ml of 5% cremophor only. Prior to injection of the test substances 5 blood samples (0.2ml each) were withdrawn through a venous catheter at 10 min intervals and 13 more samples were collected after the *iv* treatment. The chronically treated animals received the CR BNO 1055 extract for 90 days mixed with the pelleted food, which was prepared such that 100 or 400mg CR BNO 1055 per kg BW was provided (groups CR100 and CR400). Since the average weight of the animals was 330g the calculated uptake of each animal was either 33 or 133mg CR BNO 1055. The control animals received soy-free, potato protein-enriched food without any supplement. The brains of the animals were removed and frozen on dry ice. Horizontal slices (0.5mm thick) were cut with a freezing microtome and from slices containing the preoptic area (POA) and the medio-basal hypothalamus (MBH) punches were made with sharp hypodermic needles. From homogenates of the punches RNA was extracted for quantitative real time RT-PCR (Taqman 7700, Applied Biosystems) and the expression of ER-alpha, ER-beta and tyrosine hydroxylase (TH) (the rate limiting enzyme of dopamine synthesis) was quantified. For the methodological details see (6). Rats in the positive control group were treated for 90 days with estradiol-17 beta (E2, 0.5mg/20 g food)-containing food. In the basis of daily food uptake (Table 1) it could be calculated that this corresponded to 0.32mg E2/day/animal. LH, prolactin and TSH were measured by RIA for which the reagents were kindly provided by Dr. A.F. Parlow (NIAMDD program).

RESULTS

The effect of acute treatment with 62.5mg of the special extract CR BNO 1055 resulted in a reduction of serum LH levels (Fig. 1) and this is shown for one rat and compared with a control and an E_2-treated ovx rat. This indicates that compounds in this extract affect the hypothalamo-pituitary axis, which results

in less pituitary LH secretion. Direct effect of this *Cimicifuga* extract on pituitary gonadotrophs can be excluded since addition of various amounts of this extract to pituitary cell cultures did not change the LH concentrations in the culture medium (Fig. 2b). Also, TSH secretion remained unchanged (Fig. 2c). Prolactin, measured in the same culture media, was inhibited (Fig. 2a).

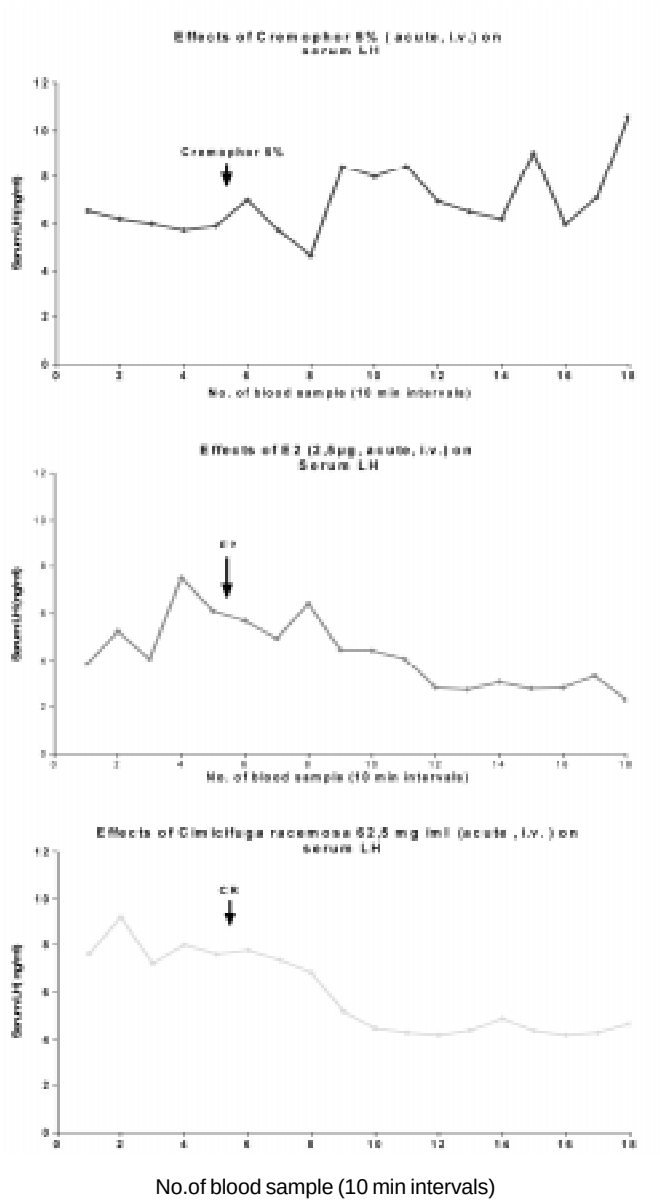

Fig. 1. Serum LH levels in ovx rats are high and significantly reduced by an acute intravenous injection of *Cimicifuga racemosa* extract BNO 1055 (CR). This LH-releasing effect is mediated in the CNS, most likely the hypothalamus, since the CR extract had no direct effect on the pituitary (see Fig. 2). Blood samples were collected at 10 min intervals.

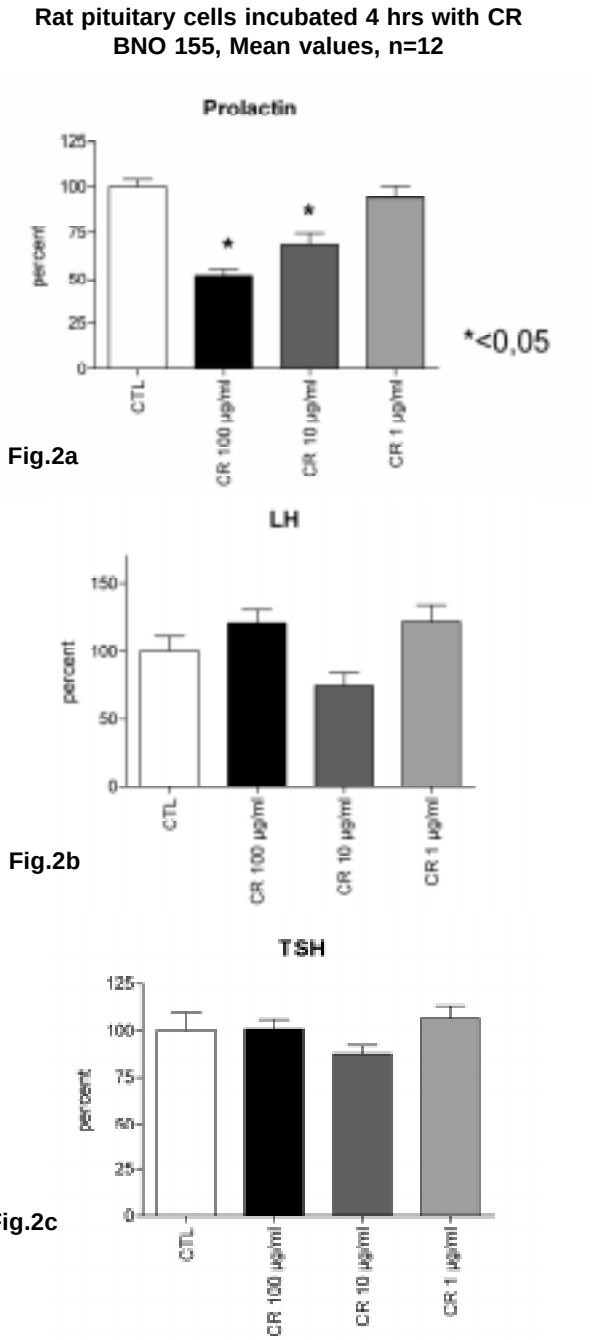

Fig. 2. Effects of 3 doses of CR extract BNO 1055 on prolactin, LH and TSH concentrations in the culture medium. Dispersed pituitary cells were pre-incubated for 24 h and then incubated for 4 h with the extract whereas the control plates were treated with solvent only. Neither LH nor TSH secretion was affected by CR, indicating no direct effect of this extract on these hormones. In contrast, pituitary prolactin release was directly inhibited by the CR extract. Prolactin release is known to be inhibited by dopamine and, therefore, the results suggest that the CR extract contained dopaminergic compounds.

As expected, and published in the literature [for reviews, see Asarian and Geary (15)], chronic E_2 treatment caused reduction of food intake significantly (Table 1, $p<0.05$) while the CR extract in the food had no effect on the food intake. In the POA and MBH the chronic application of CR extract BNO 1055 had profound effect on the expression of estrogen receptors alpha as well beta as on tyrosine hydroxylase (TH) (Fig. 3). Interestingly, in the MBH of the ovx animals the CR extract as well as E_2 (which is routinely given as a positive control treatment) there was reduced ER-beta gene expression while ER-beta gene expression in the POA was increased by both E_2 and the CR extract BNO 1055 treatments. The expression of TH gene was markedly enhanced by E_2 and also by the CR extract treatments.

DISCUSSION

The present *in vitro* and *in vivo* data explain the efficacy of CR BNO 1055 extract in reducing climacteric complaints in postmenopausal women. We demonstrated recently in one double-blind placebo-controlled and one open study that the major climacteric complaints are very efficiently reduced by CR BNO 1055 (trade names are Klimadynon ® and Menofem ®) to the same degree as under 0.6mg conjugated estrogens (11). This is most likely due to a reduction of sweating (hotflushes) episodes at night since the patients slept better and longer and this resulted in improvement of the quality of their life. The acute inhibiting effects of CR BNO 1055 on LH release in the ovx animals suggest that the extract acts either on the hypothalamus and/or the pituitary. A direct effect on the pituitary, however, was ruled out by the observation that CR BNO 1055 did not inhibit LH release from pituitary cell cultures. Interestingly, however, the black cohosh extract inhibited the release of prolactin by the lactotrophs, indicating the presence of dopaminergic compounds which address the D_2 receptor of the lactotrophs (Fig. 2a). Hence, it can be concluded that CR BNO 1055 acts on the hypothalamus to reduce LH but not TSH levels, pointing to a high specificity within the hypothalamus. Possibly, the dopaminergic compounds act to reduce prolactin release by a direct action in the pituitary and also in the hypothalamus to inhibit the activity of the GnRH pulse generator. Therefore, we studied in some detail the mechanisms which might be involved in reducing serum LH levels and were able to demonstrate that pulsatile LH release was indeed inhibited. This indicated that the activity of GnRH pulse generator was inhibited most likely by reducing the GnRH release per pulse. The GnRH perikarya of neurons in the rat are located in the medial preoptic area (POA) and their axons terminate in the medial basal hypothalamus (MBH) in the median eminence.

TH is the rate-limiting enzyme for dopamine synthesis in the hypothalamus. Therefore, its increased activity, which is suggested by the increased gene expression, may increase the dopaminergic tone within the POA and this may be one of the important mechanisms by which E_2 and CR BNO 1055 dampen the activity of GnRH pulse generator. Interestingly, the CR extract BNO 1055 also has some anti-osteoporotic effects in postmenopausal women as evidenced by decreased serum osteocalcin and serum CrossLaps (5, 11). These substances are markers of osteoblast and osteoclast activity, respectively. The mechanism by which the CR extract BNO 1055 acts in the bone remains largely unclear. We have recently shown that compounds in this extract address the arylhydrocarbon receptor (14) and this receptor is known to interact with estrogen receptor of the alpha subtype in the absence of estrogens (16). Since this estrogen receptor is primarily

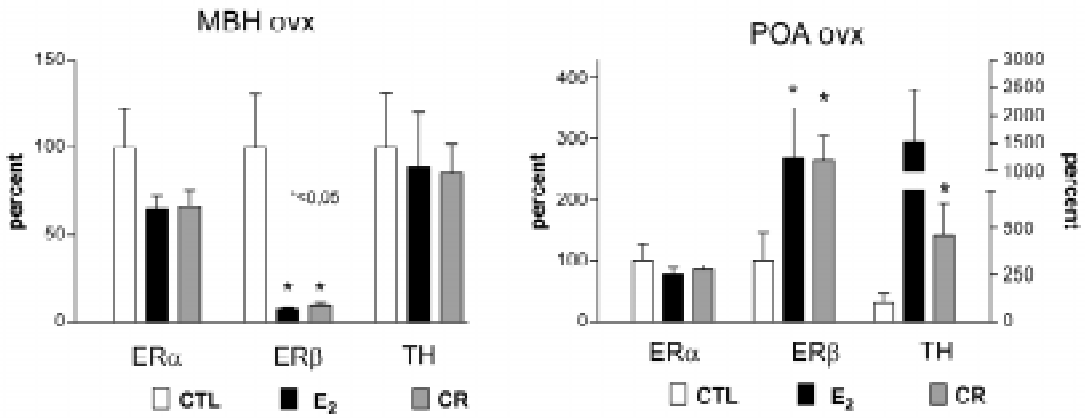

Fig. 3. Gene expression of Estrogen Receptor alpha and Estrogen Receptor beta (ER-alpha and ER-beta) and of tyrosine hydroxylase (TH) in the medio-basal hypothalamus (MBH) and preoptic area of ovariectomized rats. Both E_2 and CR had significant effects on the expression of ER-beta gene, which was inhibited in the MBH and stimulated in the POA, while TH gene expression was stimulated in the POA and this effect was significant for the CR extract.

responsible for transmitting estrogenic effects into the bone (17) this may be the mechanism by which the CR extract BNO 1055 acts in the bone. Originally, we and others speculated that CR extracts contain estrogenic compounds but in the past few years it became increasingly clear that this is not the case. None of the so far studied CR extracts binds to recombinant human ER-alpha or ER-beta protein (13) or was the growth of the ER-alpha positive human mammary cancer cell line MCF7 stimulated, which would be an estrogenic effect (18, 19).

Taken altogether, much more research needs to be done. Soy products may prove to have some anti-osteoporotic effect. They are, however, without significant effect on climacteric complaints. The isoflavones in soy products, however, have mild estrogenic effect and, therefore, their safety with respect to uterus and mammary gland needs to be established. The *Cimicifuga racemosa* extract CR BNO 1055 appears to be equipotent to conjugated estrogens in reducing climacteric complaints. All extracts studied so far have also some anti-osteoporotic effect. More research, however, is needed to understand clearly the mechanisms of action which are not estrogenic in nature but may involve neurotransmitter-like activities.

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