Effect of single neonatal treatment with the soy bean phytosteroid, genistein on the sexual behavior of adult rats

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Hormonal imprinting develops during the perinatal critical period, when the target hormone meets the yet unmatured receptor. As a consequence of imprinting the receptor accomplishes its maturation reaching the binding capacity characteristic to adults. In this period in the presence of foreign molecules similar to the target hormone faulty imprinting may occur with life-long consequences. Soy bean contains phytosteroids which can mimic estrogen effects. In the present experiments single genistein (20 µg) or combined genistein + benzpyrene (20 µg) treatments were done neonatally and the sexual behavior of male and female adult animals was studied. Genistein significantly increased the lordosis quotient of females, which was compensated by neonatal benzpyrene treatment. Genistein also enhanced the sexual activity of males, and this was significantly not reduced by parallel benzpyrene treatment.

The results show that neonatal genistein exposure can imprint sexual activity for life and the presence of a second imprinter can modify genistein’s behavioral effect.

Keywords: sexual behavior, genistein, phytoestrogen, benzpyrene, hormonal imprinting, soy bean

There is a very sensitive critical period perinatally in the connection between the developing receptor-signal transduction system and the target hormone (5). During this period the first encounter with the hormone establishes the hormonal imprinting by which the receptor accomplishes its maturation and the cell’s response develops similar
to the adult’s one (6). However, in that time the excess of the target hormone or molecules which can also bind to the not completely selective receptor (members of the same hormone family, synthetic hormone analogues, environmental pollutants with hormone-like structure etc) can cause faulty imprinting with life-long harmful consequences (7, 8). This happens with type of hormones (polypeptide or amino-acid type), nevertheless this has an outstanding role in the case of steroids (9). The faulty imprinting induced by them is manifested at receptorial, morphological, biochemical, genetic, even sexual behavioral level (2, 3, 5, 9, 13–15, 17, 23).

Genistein is a soy bean phytosteroid (isoflavone, phytoestrogen), which has been present in many foods, in the Southeast Asian diet for nearly five millenia, in the US and European diet in the 20th century (1). According to the scientific literature its use can be beneficial (it inhibits atherosclerotic plaque formation, and manifestation of osteoporosis, moreover it has an antioxidant effect, etc.) and harmful (adverse effects on reproductive organs, increases vulvar carcinoma, etc.) alike (1, 4, 21, 22, 24). Neonatal treatment with genistein caused uterine adenocarcinoma in mice and alterations of the epithelium in the excurrent ducts of rat (12, 18). Since genistein is present in soy-based infant foods, it was reasonable to study the effect of neonatal treatment with it on the sexual behavior of adult male and female rats. As an other factor, the environmental pollutant benzpyrene – which causes very strong alterations of sexual behavior after neonatal treatment (10) – can also enter into the organism of infants, we also studied the combined effect of genistein and benzpyrene.

Materials and Methods

Animals and treatment

Wistar rats of our (Charles River originated) closed breed were housed at room temperature under a normal light cycle. Food and water were available ad libitum. Newborn (within 24 h after birth) male and female rats were treated with a single dose of 20 μg genistein (Sigma, USA) or 20 μg genistein + 20 μg benzpyrene (Sigma, USA), subcutaneously. Substances were solved in 0.066% dimethylsulphoxid (DMSO) controls received the solvent (normal saline) and DMSO. The sexual behavior was tested in adult age (four months old).

Study of female animals

The receptivity of female rats was measured by the help of indicator (experienced) males. Two parameters were recorded for the evaluation of receptivity,
the Meyerson index and the lordosis quotient. The former gives a binary answer for the appearance of the lordotic response as a result of the primary mounting by males (16). The latter is a ratio of the lordosis percent in ten mountings (L/M). For comparable results the females within the two-week study were screened only during estrus (the correct timing was made by vaginal smears).

In each group five–six animals were tested a day. During the two-week testing period one animal was tested approximately four times (at 4 different days) to count a mean value for the test variables.

The average of the daily data were used for evaluating significance with Student’s “t” and $\chi^2$ tests.

In each group 24 females, 72 animals as a sum were studied.

**Study of male animals**

At first the males were studied for mounting to each other (heterotypic behavior) in a common cage (on two consecutive days for three hours a day). After that the male’s seeking behavior was studied being alone in a cage for five minutes. The suitable males were selected.

Test females were ovariectomized under ether narcosis. The tests were done two weeks after ovariectomy. Forty eight hours prior to the test 30 $\mu$g/animal estradiol monopropionate (Richter, Budapest) dissolved in sunflower seed oil was administered subcutaneously to the ovariectomized animals, while 4 hours prior to the test 500 $\mu$g/animal progesterone (Richter, Budapest) was injected subcutaneously. This procedure promotes receptivity.

Ten adult males (in each group, 30 as a sum) were tested in a 4-week period, once a week for 30 min. Five different patterns (five grade scale) of behavior were distinguished (11). Males without any mounting, intromission or ejaculation within 30 min time exposed to a receptive (hormone treated) female, were taken as inactive. Others performed only mounting and some of the males had intromission, too, without ejaculation. These were the sexually sluggish (under-active) males. Males were considered as sexually active, when the full scale of male’s copulation (mounting, intromission and ejaculation) appeared. The others had the same characteristics but multiple ejaculations. Significance was evaluated as above.

**Results and Discussion**

Soy bean phytoestrogens (isoflavones), as genistein or daidzein have both beneficial and harmful effects on animal and human organisms. They can influence the
reproductive organs, can prevent or promote breast cancer, have a significant antioxidant effect, reduce blood cholesterol level and the incidence of cardiovascular diseases. They can promote, however, the development of vulvar carcinoma, and by neonatal treatment the uterine adenocarcinoma (1, 4, 12, 18, 21, 22, 24). As the neonatal period is the time of hormonal imprinting (5–9) and soy-based infant formulas contain significant amounts of phytoestrogens (20), it was reasonable to study the effect of genistein on the sexual behavior of animals. In earlier experiments by perinatal treatment with steroid hormones or their analogues life-long faulty imprinting was provoked. Considering that the isoflavone concentration in the blood of soy-based formula fed infants could be 13,000–22,000-fold higher, than plasma estradiol concentration in this time (20), this study seemed to be extremely important. In the parallel experiments benzpyrene represented the urbanized milieu in which the infant lives.

In our experiments the influence of a single neonatal treatment with genistein or genistein + benzpyrene on the sexual behavior of adults was studied. This means that a single contact caused the alterations in the sexual behavior after 4 months. Considering this, the results seem to be impressive.

In females the Meyerson index (the response to the first mounting) shows a mild elevation in the genistein treated animals (Fig. 1), however this is not significant. At the same time there is a significant (p<0.001) elevation in the lordosis quotient (response to 10 mountings), and this elevation proved to be also significant (p<0.001) to the value of the genistein and benzpyrene treated animals (Fig. 2).

![Meyerson index](image)

Fig. 1. Meyerson index of the female animals
In males half of the control animals was inactive (Fig. 3). This inactivity significantly (p<0.001) decreased in the genistein treated animals (Fig. 4) and non-significantly elevated in the genistein + benzpyrene treated rats (Fig. 5). A significant increase could also be observed in the number of multiple ejaculations, there was no multiple ejaculation in the controls, while it was present in 10% of the genistein treated
and in 5% of the genistein + benzpyrene treated animals. The activity number based on the 5-grade scale is 1.82 in the controls, 2.40 in the genistein treated and 1.97 in the combined treated animals.

**Activity**

*Genistein*

![Pie chart](image1)

**Activity**

*Genistein-benzpyrene*

![Pie chart](image2)

Fig. 4. Sexual activity (in percents) of neonatally genistein treated male animals. Ejacs = multiple ejaculations

Fig. 5. Sexual activity (in percents) of neonatally genistein + benzpyrene treated male animals. Ejacs = multiple ejaculations
The results unequivocally demonstrate that a single neonatal genistein treatment promotes sexual activity of adult rats in both sexes. However, the environmental pollutant benzpyrene compensates this effect in females. Considering that neonatal benzpyrene exposure alone radically reduced adult’s sexual activity in previous experiments (10), the result is clearly understandable. Turning over the problem and project it to human relations: benzyrene pollution is always present in urbanized areas and this can reduce later sexual activity, which can be compensated by soy bean containing infant food.

Our results demonstrated the sexual behavioral effect of a single neonatal treatment in adult age. However, it is not sure that continuous treatments (feeding) has the same role. In case of another phytoestrogen, coumestrol, Whitten et al. (25, 26) found that neonatal exposure of pups through milk of rat dams, or chow diet during the first 10 days or through the 21 days of lactation caused deficits in male sexual behavior (reductions in mounting and ejaculation frequency), which is the opposite of our results. Santti et al. (19) also demonstrated diethylstilbestrol like effects on male sexual behavior after neonatal phytoestrogen treatment. This means that there could be differences even in the direction of the effect according to the manner of exposure, however, the phytoestrogen exposure causes imprinting for life in each case.

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