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Effect of tamoxifen treatment at adolescent age on the sexual behaviour and steroid hormone receptor binding of adult female rats

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Hormonal imprinting takes place perinatally, at the first encounter between the target hormone and its developing receptor. However, there is a secondary critical period of imprinting at puberty. In these periods molecules similar to the hormones (members of the same hormone family, antagonists, certain environmental pollutants, etc.) can cause faulty imprinting with lifelong consequences. In the present experiments 5+2 days of tamoxifen treatment (120 μg/day) at adolescent age dramatically (from approx. 40% to 10%) reduced the sexual activity (Meyerson index and lordosis quotient) of female rats, soon after the finishment of the treatment and between four to six weeks after treatment. Similar results were observed in animals neonatally treated with allylestrenol and tamoxifen treated at puberty. Thymic glucocorticoid receptor and uterine estrogen receptor binding capacity were not influenced.

Keywords: estrogen receptors, glucocorticoid receptors, hormonal imprinting, sexual behavior, Tamoxifen

Hormonal imprinting takes place perinatally, when the target hormone meets the developing receptor (4–6). As a consequence of imprinting the receptors reach the binding capacity which is characteristic to the adult age (7, 8), however without imprinting this binding capacity remains weaker (10). In the critical imprinting period molecules resembling to the target hormone (hormone analogues, members of the same hormone family, environmental pollutants with hormone-like character, antihormones,

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etc.) can cause faulty imprinting with lifelong disturbed receptor binding and response of the receptor bearing cell (1–3, 7–9, 17, 18, 20, 25). However, though in case of steroid hormones the first days of life are the postnatal critical periods, in some cases there is a possibility for provoking imprinting in adolescents, as it was demonstrated by using nandrolone, the administration of which developed a decrease of thymic and uterine receptor binding capacity (density) in adult age (11).

Tamoxifen is a synthetic non-steroid antiestrogen (e.g. in case of breast), which sometimes (mainly in skeletal and cardiovascular tissues) mimics estrogen effects (19, 21). Single neonatal treatment with tamoxifen practically nullifies sexual response to males in adult female animals (12) and decreases the binding capacity of adult's thymic glucocorticoid and uterine estrogen receptors (13). These observations make reasonable to study the effect of pubertal tamoxifen treatment on the behavioral and receptorial parameters. Considering that investigations on animals which were imprinted with allylestrenol – a progestagene steroid, which was (is) used for protecting endangered pregnancies – have a practical use, we also studied the tamoxifen effect on that kind of animals.

Materials and Methods

Animals and treatment

Newborn female rats of our closed bred Wistar strain were treated with 17.5 μg allylestrenol (Richter, Budapest, Hungary) suspended in 0.2 ml sunflower seed oil, within 24 h after birth, subcutaneously. Controls were treated with the oil only. When the animals were ten weeks old, tamoxifen (Sigma, USA, 60 μg/day) treatment was administered for 5+2 days (during week-end the animals was not treated) and 24 h after the last treatment the females were tested for sexual behavior. Two weeks after the finishment of the first testing (4 to 6 weeks after finishing the tamoxifen treatment) the testing was repeated. When the animals were 5 and a half months old, uteri and thymi were studied for receptors 8 days after ovariectomy. Three thymi or four uteri were homogenized for winning one preparation for one measurement. All assays were performed in duplicate. Data (means) resulted from measurements of 3–5 different homogenates in each group.

Sexual-behavioral 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 on the first mounting of the male (22). 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.

The average of the daily data were used for evaluating significance with Student *t*- and χ^2-tests.

Preparation of cytosol fraction for receptor assays

All procedures were performed at ice/water temperature. Tissues examined were cut into pieces and homogenized in Tris-HCl buffer-containing 1.5 mM EDTA, pH 7.4 (freshly supplemented with 20 mM molybdate and 2 mM dithiotreitol) with a motor driven glass-teflon Potter homogenizer 1.5 ml/g wet weight. Homogenates were centrifuged at 100,000 g for 60 min at 4 °C and the supernatants were used for receptor assays. Protein content was estimated by Coomassie-blue method.

Glucocorticoid receptor – thymus cytosol

500 μg protein was incubated with 10, 5, 2.5, 1.25, 0.6, 0.3 and 0.15 nM ^{3}H-dexamethasone (Amersham, Buckinghamshire, England; spec. act. 1.8 TBq/mmole) in the absence or in the presence of 1000-fold molar excess of unlabeled ligand (Sigma, USA) in a total volume of 100 μl at 0 °C for 18 hours. Bound glucocorticoid was separated by the charcoal method and counted in OptiPhase, HiSafe (Pharmacia, Lund, Sweden, 35% efficiency). Radioactivity measured in the presence of 1000 nM dexamethasone was regarded as nonspecific binding.

Estrogen receptor – uterus cytosol

300 μg cytosolic protein was incubated with 5, 2.5, 1.25, 0.62, 0.31, 0.15 and 0.07 nM 2,4,6,7-^{3}H-estradiol (Izinta, Budapest, Hungary, 3.2 TBq/mmole spec. activity) in the absence or in the presence of 1000-fold molar excess of unlabeled ligand (Organon, Oss, Holland). Condition of incubation, termination of the reaction and counting were identical to those of receptor assay on thymus cytosol.

Analysis of receptor-results

Analysis of results were carried out by the computer program EBDA and LIGAND written by McPherson (23, 24). EBDA was used to process raw data.

LIGAND (non-linear curve fitting program) was used to obtain final parameter estimates. Statistical analysis of the final parameters was calculated by the computer program DATAANALYSIS V.1.0. Statistical and Design Services, 1985; analysis of variance, simple F-test comparison.

Results and Discussion

Tamoxifen is a synthetic non-steroid molecule with high affinity to estrogen receptors. It can antagonize estrogen effect in some cases, in other cases it is agonistic (19, 21). Given neonatally to females in a single dose, it provokes imprinting with a subsequent decrease of thymic glucocorticoid and uterine estrogen receptor binding capacity (13). In addition it dramatically decreases the sexual activity (12). Considering that perinatal treatment with tamoxifen causes an immediate neuronal loss in the sexually dimorphic nucleus of the preoptic area (26) and defeminizes female's corpus callosum (16), the effect on sexual activity is understandable. These facts made reasonable to study the imprinting effect of tamoxifen treatment in the secondary critical period, at late adolescence (2).

Allylestrenol (Gestanon, Organon), which is used in human therapy for protecting endangered pregnancies, in animal experiments causes decrease of receptor binding capacity (14) and an extreme loss of sexual activity (15) in adult rats after perinatal imprinting. Since many pregnant women had been treated with this drug it seemed to be plausible to study the combined (allylestrenol and tamoxifen) effect in our animal model.

In the present experiments the Meyerson index as well, as the lordosis quotient of pubertally tamoxifen treated animals were significantly ($p<0.01$) less (from 40% decreased to about 10%) than those of the controls (Figs 1 and 2), soon after the treatment.

Table I

Saturation analysis of the thymic glucocorticoid and uterine estrogen receptors (±SD) of perinatally allylestrenol treated and non-treated; and at adult age tamoxifen treated and non treated adult female rats

Group	Receptor	K_d M^{-10}	B_{max} M^{-10}
Control	estrogen	9.08 ± 0.87	7.37 ± 1.27
TAM-treated	estrogen	7.42 ± 0.87	6.91 ± 1.84
ALL+TAM treated	estrogen	8.78 ± 4.55	7.13 ± 3.47
		K_d M^{-9}	B_{max} M^{-9}
Control	glucocorticoid	8.90 ± 3.42	3.36 ± 1.39
TAM-treated	glucocorticoid	10.60 ± 1.40	3.17 ± 0.75
ALL+TAM treated	glucocorticoid	12.90 ± 0.14	3.91 ± 0.34

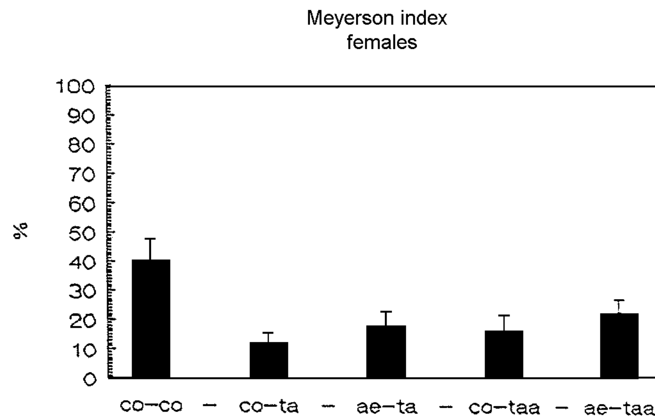

Fig. 1. Meyerson index of female rats, neonatally allylestrenol treated or not treated and tamoxifen treated or not treated at puberty. co=control; ta=tamoxifen treated; ae=allylestrenol treated; taa=second observation four to six weeks after the end of pubertal treatment

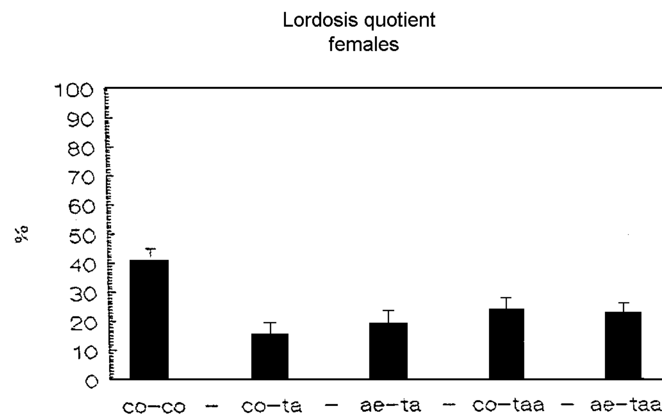

Fig. 2. Lordosis quotient of female rats, neonatally allylestrenol treated or not treated and tamoxifen treated or not treated at puberty. co=control; ta=tamoxifen treated; ae=allylestrenol treated; taa=second observation four to six weeks after the end of pubertal treatment

Similar results had been observed in the neonatally allylestrenol treated and later tamoxifen treated animals ($p<0.01$), without significant difference to the only tamoxifen treated females. Between four and six weeks after the end of tamoxifen treatment similar results were won, which calls attention to the prolonged behavioral effect of tamoxifen imprinting.

The saturation analysis of the thymic glucocorticoid and uterine estrogen receptors did not demonstrate significant differences (to control) after pubertal tamoxifen treatment or after neonatal allylestrenol and pubertal tamoxifen treatments in the affinity or density of receptors (Table I) in the five and a half months old animals. This means that in the adolescence different receptors (brain, thymus and uterus) of the same hormone (estrogen) are differently influenced by tamoxifen imprinting and brain receptors retained their sensitivity, while the "peripheral" receptors lost it. It seems to be likely that this sensitivity is also imprinter-dependent, since in earlier experiments (11) nandrolone provoked imprinting of the "peripheral" receptors.

As it was demonstrated, neonatal allylestrenol treatment did not influence the tamoxifen effect on the sexual behavior, there was not a sum of the negative influences. Moreover, pubertal tamoxifen treatment compensated the negative effect of perinatal allylestrenol treatment (demonstrated in earlier experiments (14) on the receptor's binding capacity.

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