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PII S0024-3205(98)00301-4

Life Sciences, Vol. 63, No. 6, pp. PL 101-105, 1998
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0024-3205/98 $19.00 + .00

PHARMACOLOGY LETTERS
Accelerated Communication

NEONATAL VITAMIN E TREATMENT INDUCES LONG TERM GLUCOCORTICOID RECEPTOR CHANGES: AN UNUSUAL HORMONAL IMPRINTING EFFECT

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(Submitted March 31, 1998; accepted April 23, 1998; received in final form May 20, 1998)

Abstract. Single neonatal vitamin E treatment significantly altered the affinity (K_d) of thymic glucocorticoid receptors in male adolescent and adult rats. In six weeks old animals the affinity increased (and there is a tendency for an increase in receptor density), in twelve weeks old animals the affinity decreased. The thymic glucocorticoid receptors and uterine estrogen receptors of female animals were not influenced at all. Thousandfold tocopherol did not compete with labeled dexamethasone for their receptors, suggesting that neonatal vitamin E imprinting effect was not done at direct receptorial level. © 1998 Elsevier Science Inc.

Key Words: hormonal imprinting, perinatal treatments, tocopherol, thymic glucocortcoid receptor

Introduction

When the developing hormone receptor at first meets the hormone in the perinatal critical period a hormonal imprinting occurs, which influences the further fate of the receptor-hormone connection for life (1-3). In case of the encounter with the appropriate hormone a normal imprinting develops which is absolutely needed for the normal completion of receptor maturation (4). In case of the encounter with molecules different from the adequate hormone, however able to bind to the developing receptor, a faulty imprinting takes place which lifelong modifies (increases or reduces) the hormone binding capacity of the receptor and as a consequence of this, the response of the receptor bearing cell (3,5).

The excess of some of the hormones bound by the steroid receptor superfamily as well, as presence of related molecules (synthetic steroid hormones, environmental pollutants etc) in the perinatal period can overlap on the related receptors causing misimprinting which is later manifested in changes of hormone binding, sexual hormone production and sexual behavior as well (3,5), as in alterations at genetic (6) and morphological levels (7-9). Similar events can also be observed after neonatal vitamin A or D treatment (10,11). These vitamins also have receptors in the steroid receptor superfamily. Taking the above into consideration as well as the lipid solubility of vitamin E and its effects on sexual parameters (12), it seemed reasonable to investigate the possible imprinting effect of tocopherol.

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Methods.

Newborn (before 24 h after birth) Wistar rats of our closed breed were injected subcutaneously with 1.5 mg/0.05 ml/animal tocopherol acetate (Vitamin E, Richter, Budapest). Controls received the vehicle (sunflower seed oil). Thymus of 6 and 12 weeks old male and female animals and uterus (8 days after ovariectomy) of 12 weeks old female animals were removed for receptor kinetic analysis. For each measurement three thymi and five uteri were pooled. Each measurement was done in duplicate.

Preparation of cytosol fractions. All procedures were performed at ice/water temperature. Tissues (1.5 ml/g wet weight) 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. Homogenates were centrifuged at 100.000g for 60 min at 4 °C and the supernatants were used for receptor assays. Protein content was estimated by the Coomassie blue method.

Glucocorticoid receptor assay for thymus cytosol. Five hundred micrograms of protein was incubated with 10, 5, 2.5, 1.25, 0.6, 0.3 and 0.15 nM ^{3}H-dexamethasone (Amersham, Buckinghamshire, UK; spec. act. 1.8 Tbq/mmol) in the absence or presence of 1000-fold molar excess of unlabeled ligand (Sigma, Mo. USA) in a total volume of 100 µl at 0 °C for 18 h. 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-fold molar excess of unlabeled ligand was regarded as nonspecific binding.

Estrogen receptor assay for uterus cytosol. Three hundred micrograms of cytosolic protein was incubated with 5 nM 2,4,6,7-^{3}H-estradiol (Izinta, Hungary, 3.2 Tbq/mmol spec. activity) and increasing concentrations of estradiol (0, 2, 5, 10, 20, 50 and 1000 nM, Organon, Oss, Holland). Conditions of incubation, termination of the reaction and counting were identical to those of receptor assay on thymus cytosol.

Analysis of the results were carried out by the computer program EBDA and LIGAND written by McPherson (13,14); EBDA was used to process raw data. Ligand (non-linear curve fitting program) was used to obtain final parameter estimates. Statistical analysis of final parameters was calculated by the computer program DATAANALYSIS, v.1.0 (analysis of variance, simple F-test comparison)..

In separated experiments the thymic ^{3}H-dexamethasone binding of untreated neonates and adults was studied in the presence of 1000-fold unlabelled tocopherol. The method was the same as given above.

Results and Discussion.

A single neonatal tocopherol treatment significantly influenced the affinity (K_d) of glucocorticoid receptors (Table 1 and Fig.1 and 2) of 6 or 12 weeks old male animals. Although the direction of changes caused by neonatal imprinting usually do not change the direction through life, present results show receptor affinity increases at 6 weeks and receptor affinity decreases at 12 weeks. A similar change in direction was previously shown under the effect of neonatal exposure to allylestrenol (15) or vitamin D_3 (16).The sex-dependence of the imprinting mechanism was also revealed in the present study, similarly to that previously shown for neonatal vitamin D_3 treatment (16).

Table 1

Effect of neonatal tocopherol treatment on the thymic glucocorticoid receptor binding affinity and capacity of adult male rats (10^{-9} M, means+/-SE; 3 animals' organs pooled in each measurement).

Group	n	age	Kd male	Bmax	Kd female	Bmax
control	**2**	6 weeks	3.97+/-0.52	2.45+/-0.24	3.76+/-0.60	4.97+/-0.82
treated	**4**	6 weeks	2.08+/-0.11*	3.29+/-0.86^{+}	3.59+/-0.44	4.62+/-0.25
control	**5**	12 weeks	3.24+/-0.75	3.73+/-0.16	3.19+/-0.99	3.03+/-0.53
treated	**5**	12 weeks	4.40+/-0.55*	3.57+/-0.86	2.94+/-0.90	3.27+/-0.82

* = $p<0.03$; $^{+}$ = $p=0.55$

The imprinters used in earlier experiments either have receptors in the steroid receptor superfamily, or they are structurally similar to steroids (3,5) . However, there is no structural similarity in the case of vitamin E and in our present knowledge its receptor - if it exists at all- does not belong to the family mentioned. There are sparse data on vitamin E receptors. Catignani (17) demonstrated a specific receptor-like binding

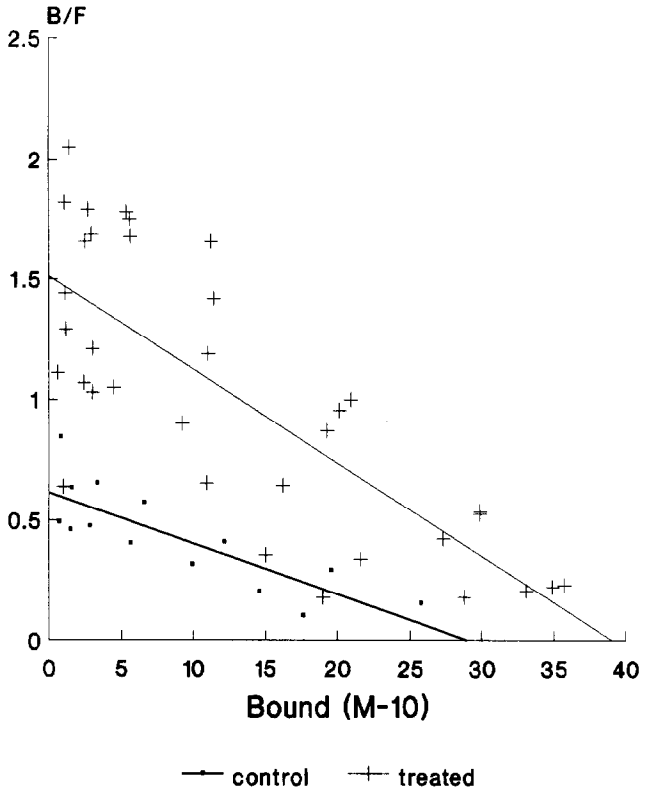

Fig.1. Scatchard plots of 6 weeks old animals. Graphical representation of data of saturation analysis processed by the program EBDA.

protein in rat liver cytoplasm, however its nature was not cleared. Donchenko et al. (18) found a tocopherol binding protein in the nuclear matrix of the liver cells. Kitabchi et al. (19) also demonstrated a receptor for tocopherol, however it was localized in the plasma membrane and this was supported by the results of Bellizzi et al. (20) who demonstrated the binding of tocopherol to the surface of erythrocytes. This means that receptor could be present, however its localization as well, as its classification to some known receptor family is uncertain. Nevertheless, in our present experiments tocopherol was not able to abolish in vitro dexamethasone binding of neonatal or adult glucocorticoid receptors, even in thousand-fold concentration. This contradicts to the steroid receptorial level imprinting effect of the neonatal tocopherol treatment and permits the suspicion of some indirect influence. However this influence must be very intensive (possibly at the gene level -6), considering the durable change of receptor affinity. Further, the present results are in agreement with an earlier proposition (9) that hormonal imprinting can be induced by agents that do not display hormone action.

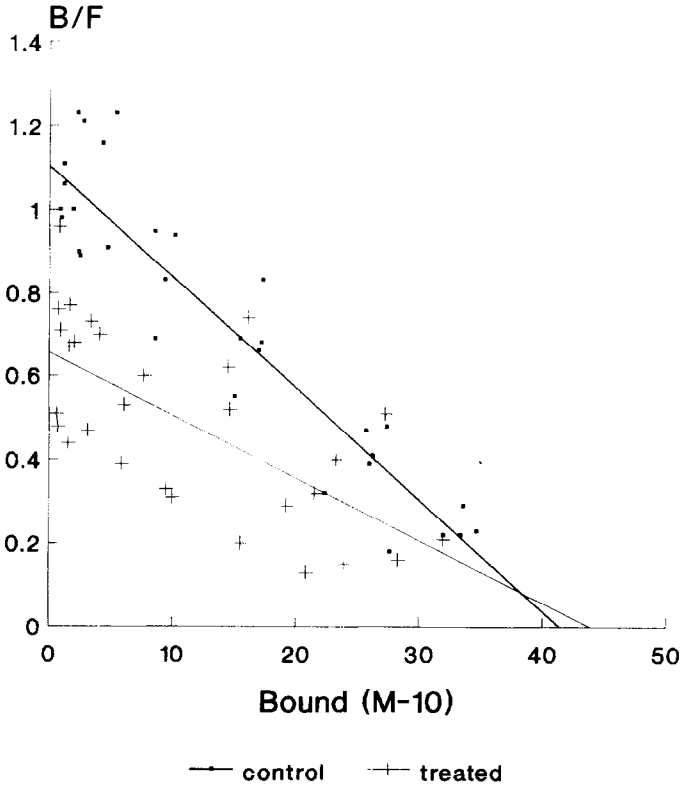

Fig.2. Scatchard plots of 12 weeks old animals. All values (details) are given in Table 1.

The uterus of twelve weeks old rats was insensitive to the neonatal tocopherol treatment (K_d=2.53+/-0.66 to 2.21+/-1.35, 10^{-9} M; B_{max}=4.47+/-0.16 to 4.23+/-0.64, 10^{-9} M in control and treated animals, respectively). This points to a difference in the sensitivity of different organs (steroid receptors) to the the effects of perinatal exposure

to imprinting inducing agents, similarly as earlier described for a combined imprinting with vitamin A, vitamin D_3, allylestrenol or benzpyrene (21), or for the transgenerational effect of benzpyrene imprinting (22).

Though the experiments neither answer the question on the hypothetical vitamin E receptor, nor the causes of vitamin E effects on sexual parameters (12), they call attention to the long-lasting effect of a single perinatal tocopherol treatment at steroid receptor level, which has to be considered in case of maternal or neonatal treatments.

Acknowledgements

This work was supported by the National Research Fund (OTKA) T-017775, Hungary. The authors thank Ms. Katy Kallay for the experienced technical work.

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