Effect of a Single Neonatal Treatment with Steroid Hormone or Steroid-Like Molecules on Myocardial Ouabain Binding in the Adult Rat

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Abstract. A single neonatal treatment of rats with vitamin D_3, gibberellin, allylestrenol or diethylstilbestrol (DES) influenced the ouabain binding capacity of myocardial Na, K-dependent ATP-ase. Of the active molecules tested, vitamin D_3, DES and gibberellin had appreciable impact on myocardial ouabain receptors, enhancing and depressing their activity, respectively. The thymic dexamethasone and uterine estrogen receptors did not alter their binding capacity in response to neonatal exposure to vitamin D_3 or gibberellin.

Key words: Vitamin D_3 — Gibberellin — DES — Neonatal imprinting — Ouabain binding

Introduction

In the perinatal period, the primary interaction between a hormone and its target cell gives rise to hormonal imprinting, which accounts for the maturation and functional normalization of the receptor (Csaba 1980, 1981). If in that critical period of the receptor development molecules structurally slightly different from the respective hormone, yet capable of binding to its receptor, are present in an appropriate concentration, their interaction with the receptor alters the future binding capacity thereof and consequently also the response of the cell (Csaba 1984, 1986).

Earlier studies along this line have shown that a single neonatal treatment with a synthetic steroid hormone, such as dexamethasone (Inczefi-Gonda and

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Csaba 1985), or allylestrenol (Csaba et al. 1985), or with a steroid-like molecule capable of binding to a steroid receptor, such as diethylstilbestrol (DES) (Csaba et al. 1985) or benzpyrene (Csaba and Inczefi-Gonda 1984) accounted for a lasting decrease in the number of steroid binding sites. At the same time, neonatal treatment with a steroid (triamcinolone) influenced not only the steroid receptors, but also the binding capacity of the myocardial (Na, K-ATPase) receptor for ouabain which has a steroid-like structure (Inczefi-Gonda et al. 1986a). On the other hand, neonatal exposure to ouabain had no influence on steroid receptor activity.

We performed experiments on rats to examine the impact of a single neonatal exposure to the chemically steroid-like vitamin D_3 and the plant hormone gibberellin on thymic dexamethasone binding, uterine estrogen binding and myocardial ouabain binding. Some groups of the experimental animals were treated with allylestrenol and DES, which are known to damage the steroid receptors on neonatal exposure (Csaba et al. 1985), to investigate the influence on myocardial Na, K-dependent ATPase activity.

Materials and Methods

Newborn CFY rats of both sexes were treated within 24 h of birth with a single subcutaneous dose of gibberellin (Fluka, Switzerland; 5 μg/0.1 ml saline/animal) or vitamine D_3 (Chinoin, Hungary; 10,000 IU in 0.5 ml oil), into the cervical region. The control rats received similar injections of saline. At 2 months of age, cytosolic preparations obtained from rat thymus, and from the uterus of the gibberellin-treated females, were examined for ^{3}H-dexamethasone binding and ^{3}H-estradiol binding, respectively, and membrane preparations obtained from the heart muscle were examined for ^{3}H-ouabain binding. Heart muscle cell membrane preparations were also obtained from female rats neonatally treated with allylestrenol (Richter, Hungary; 17.5 μg/rat) or diethylstilbestrol (Richter, Hungary; 8.8 μg/rat).

Cytosolic preparations

The thymic glands of 5 – 6 rats of both sexes, or uteri of 8 – 10 females were homogenized in 1.5 volumes of ice-cold 1.5 mmol/l EDTA and 1.0 mmol/l dithiothreitol containing 10 mmol/l Tris-HCl buffer at pH 7.4, in a teflon-coated glass jar (Potter homogenizer). The homogenizate was centrifuged at 100,000 × *g* for 1 h at 4 °C. The cytosolic preparation so obtained was examined for protein content with the Coomassie-blue technique, and saturated for studying ^{3}H labeled hormone binding in three replica experiments, using the method of Clark et al. (1979) for uterine, and that of Arányi and Náray (1980) for thymic preparations. The results were evaluated by Scatchard analysis.

Hearth muscle preparations

Myocardial preparations were obtained from 20 control rats and 25 experimental rats each on three replica series as described by McNamara et al. (1974). The protein content of the preparations was determined by the Lowry technique (1951).

Determination of ^{3}H-ouabain binding

On the basis of preliminary experiments aimed at determining the optimal (saturation) concentration of labeled and unlabeled ouabain, following concentrations of drugs were used in the present

experiments: 2×10^{-8} mol/l ^{3}H-ouabain (Amersham; spec. act. 1.37 TBq mmol.l^{-1} and 8.8×10^{-4} mol/l non-labelled ouabain; experiments were performed using the method proposed by Khatter and Hoeschen (1982). Specific binding was assessed by subtracting the scintillation counts of 8.8×10^{-4} mol/l of non-labeled ouabain from those of the labeled drug.

Results

Ouabain binding was significantly enhanced by neonatal treatment of animals with vitamin D_3 or gibberellin. Treatment of animals with DES resulted in a significantly lowered ouabain binding in adult animals (Table 1). Ouabain binding in allylestrenol treated animals was enhanced, the differences as compared to nontreated controls were however insignificant. Neither vitamin D_3, nor gibberellin influenced thymic dexamethasone or uterine estradiol binding.

Table 1. Effect of neonatal treatment of rats with steroids and steroid-like molecules on myocardial ouabain binding in adult animals

Test compound	^{3}H-ouabain binding to the myocardial cell membrane femtomol/mg protein		per cent difference from control
	control S.E.	experimental S.E.	
Vitamin D_3	176 ± 36.6	*299 ± 49.7	+68
Gibberellin	216 ± 31.5	*302 ± 26.3	+39
DES	173 ± 39.8	*92 ± 64.0	−47
Allylestrenol	173 ± 39.8	200 ± 49.6	+15

* = Significance related to control: $p < 0.001$

Discussion

Earlier experimental studies have suggested that not only the respective hormone, but also structurally similar molecules capable of binding to the hormone receptor can give rise to imprinting of the latter during the critical perinatal period (Csaba 1981, 1984). For example, the structurally steroid-like molecule of benzpyrene altered the glucocorticoid binding capacity (dexamethasone) of rat thymus (Csaba and Inczefi-Gonda 1984) and neonatal exposure to DES or allylestrenol decreased the number of uterine estrogen receptors by faulty imprinting (Csaba et al. 1985). Of the two foreign molecules used in the present study, vitamin D_3 has a distinctly steroid-like structure, whereas gibberellin, although being synthesized in a steroid-like manner, is hardly steroid-like in its final form. Since neonatal exposure to these hormone-like molecules had no

influence whatever on the steroid (thymic glucocorticoid and uterine estrogen) receptors, it seems that in the perinatal period these molecules failed to bind to the steroid receptor and to alter it either qualitatively or quantitatively. As to the other two molecules tested, neither allylestrenol nor DES were examined for neonatal influence on the steroid receptors, for it has been known from earlier experiments along this line that both drugs extensively reduced the number of the uterine estrogen receptors (Csaba et al. 1985); allylestrenol also depressed thymic glucocorticoid (Inczefi-Gonda et al. 1986b) binding. Since in earlier studies ouabain, which is even less steroid-like in structure, also failed to alter the binding capacity of the steroid receptors (Inczefi-Gonda et al. 1986a), whereas the more steroid-like benzpyrene did affect it (Csaba and Inczefi-Gonda 1984), there is reason to postulate that the steroid receptor is greatly protected against adverse influence already in the perinatal period, and is therefore available for those molecules only which are structurally closely related to the respective hormone. However, Na, K-dependent ATPase, which represents the membrane receptor for ouabain, behaved differently.

The ouabain binding capacity of Na, K-dependent ATPase was considerably changed by neonatal exposure to steroid-like molecules. It was shown earlier that while the synthetic fluorinated steroid hormone triamcinolone altered the myocardial ouabain binding capacity of rats upon neonatal exposure, neonatal ouabain treatment had no similar effect on triamcinolone binding (Inczefi-Gonda et al. 1986a). All the four active molecules tested in the present study influenced the ouabain receptor when given to neonatal rats (although one of them insingificantly). Na, K-dependent ATPase seems to be sensitive to steroids or steroid-like molecules during the perinatal period, as judged from alterations of ouabain binding in adult animals. However, the steroid-like molecules tested differed in their qualitative and quantitative effects on ouabain receptor activity.

These observations substantiate our earlier suggestion (Csaba 1980, 1981, 1984, 1986) that the receptors are sensitive to "foreign" molecules during the perinatal period. However, the sensitivity may vary greatly with the nature of the receptor concerned. Whereas steroid receptors can be damaged mainly by synthetic steroids or structurally similar molecules, the ouabain receptors are equally sensitive to the adverse effects of steroids and steroid-like non-hormones. Taking into consideration that the steroid receptors have evolved for interactions with endogenous molecules, whereas the ouabain receptor Na, K-ATPase although biologically being an enzyme rather than a receptor presents a binding structure for an exogenous molecule, the greater sensitivity of this enzyme to faulty imprinting can be ascribed to its different biological nature.

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