PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 71 (1999) 133–137

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

7β-OH-DHEA counteracts dexamethasone induced suppression of primary immune response in murine spleenocytes

I. Šterzl[a], R. Hampl[a,*], J. Šterzl[b], J. Votruba[b], L. Stárka[a]

[a]*Institute of Endocrinology, Národni 8, 11694 Praha 1, Czech Republic*
[b]*Institute of Microbiology, Department of Immunology and Gnotobiology, Academy of Sciences of the Czech Republic, Praha, Czech Republic*

Received 12 March 1999; accepted 17 August 1999

Abstract

The effect of dexamethasone and of three potential antiglucocorticoids, namely dehydroepiandrosterone (DHEA) and its 7α- and 7β-hydroxylated metabolites, on primary immune response has been studied by measuring the number of plaque forming cells (NPFC) and their viability in a cell culture of murine spleenocytes. As expected, dexamethasone suppressed considerably the NPFC as well as their viability. Surprisingly, DHEA as well as its 7α-hydroxylated metabolite decreased significantly the NPFC, while the effect of 7β-hydroxy-DHEA was different: at low doses it decreased the NPFC, but this effect was less pronounced at higher concentrations. In addition, 7β-hydroxy-DHEA was able to counteract the effect of dexamethasone on the NPFC. None of the natural steroids affected the cell viability.

Keywords: Dexamethasone; Dehydroepiandrosterone; 7-Hydroxylated metabolites of dehydroepiandrosterone; Primary immune response; Murine spleenocytes

1. Introduction

Recent data from in vitro as well as in vivo experiments show that dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) may act at various levels as immunomodulatory and immunoprotective agents [1–3]. These effects cannot be ascribed only to gonadal steroids for which DHEA/S serves as a precursor [4]. Many of these effects, but not all, can be explained by nongenomic antiglucocorticoid action of DHEA/S [5,6]. It should be emphasised that no intracellular receptors for DHEA/S have been found, nor does this steroid compete for glucocorticoid receptors [7].

Unconjugated DHEA, rather than DHEAS, undergoes extensive metabolism in the liver and in other peripheral tissues [1]. Among its products a nonnegligible portion represent 7α- and 7β-hydroxylated metabolites [1], so far believed to be the only degradation products, devoid of any biological activity. Recently, evidence has been brought that these metabolites may act as locally active agents, responsible for at least some of the autocrine and paracrine immunomodulatory and antiglucocorticoid effects ascribed to DHEA/S. This opinion was based on following observations (for review until 1997 see [8]):

- 7-Hydroxylating cytochromes were restricted to the cells producing 7α- or 7β-hydroxylated metabolites. Human peripheral blood monocytes (PBMC) did not produce 7-hydroxysteroids while stromal epithelial cells isolated from human tonsils did. When PBMC were coincubated with memory T-cells and the antigen (human tetanus vaccine), addition of 7α-OH-DHEA increased significantly the formation of specific antibodies to the antigen and counteracted also the immunosuppressive effect of glucocorticoids. This indicates that PBMC were targets for 7-hydroxysteroids [8].
- In tissue cultures of rat brain astrocytes, known to

* Corresponding author. Tel.: +420-2-24905-289; fax: +420-2-24905-325.
E-mail address: rhampl@endo.cz (R. Hampl).

0960-0760/00/$ - see front matter
PII: S0960-0760(99)00134-X

metabolise DHEA and other neuroactive steroids to androgens and estrogens, when the cell contact was increased above confluence resembling thus the situation at inflammation, the metabolism was shifted to the formation of 7α-hydroxylated products on the expense to their oxidation (pregnenolone to progesterone and DHEA to androstenedione) [9].

- Dexamethasone induced the 7α-hydroxylation of DHEA in another tissue, namely in human adipose stromal cells [10].
- Direct effect of 7-hydroxylated C_{19} and C_{21}-steroids on some parameters of the immune response or their ability to counteract the action of glucocorticoid has been reported: when murine thymic cells were cultivated in presence of 7α-OH-DHEA, the dexamethasone-induced apoptosis was decreased [11].
- In an experiment with cultured murine spleenocytes DHEA surprisingly even suppressed concavalin A- or lipopolysacharide-induced cell proliferation, while one of its 7-hydroxylated metabolites, namely 5-androstene-3β,7β,17β-triol stimulated it. The latter steroid, in contrast to DHEA, also counteracted the effect of cortisol on Con A-activated lymphocyte proliferation as well as glucocorticoid-induced Il-2 and IL-3 production [12,13]. The effect of 7-OH-DHEA was not investigated in the latter studies.

The mechanism(s) through which 7-hydroxylated metabolites of DHEA act is unknown. We have studied the effect of 7α-OH-DHEA on early stages of dexamethasone action in vitro. Likewise DHEA, 7α-OH-DHEA did not compete for glucocorticoid receptors in rat hepatocytes, either influenced the activation of glucocorticoid–glucocorticoid receptor complex or its binding to DNA-cellulose in cell-free system [14].

In this paper the effect of both 7-OH-DHEA isomers on primary immune response is reported in a cell model, similar to that used by others [12,13].

2. Experimental

2.1. Steroids and reagents

Dehydroepiandrosterone, dexamethasone, HEPES and a collection of essential amino acids were purchased from Sigma (St. Louis, MO). 7α- and 7β-OH-DHEA were prepared by the method of Starka [15]. MEM, calf fetal serum and antigen were from USOL (Praha, Czech Republic).

2.2. Determination of primary immune response

Primary immune response was measured by counting of a number of plaque forming cells, (NPFC) following activation with an antigen as described previously [16]. In brief, murine spleen lymphocytes from BALBc mice, 10^7 cells/ml were cultivated in the Marbrook system consisting of a supplemented MEM medium with 10% of selected fetal calf serum mixed with the antigen (10^7 sheep erythrocytes) in the absence or presence of increasing doses of tested steroids, in an atmosphere of 96% air and 4% CO_2,, at 37°C, for 5 d. Immune response in controls (without steroids) and in experiments with tested compounds were determined as NPFC in agarose by a drop modification of the Jerne method [17]. The effect of steroids was related to the mean value of 28 control experiments, taken as 100%. The viability of the cells after cultivation was assessed by staining with 1% nigrosin (when more than 50% of the cells were stained, the toxicity of tested compounds was considered positive).

2.3. Statistics

Since the effect of steroids on NPFC was expressed in per cents of NPFC in control experiments, a careful statistical analysis of control values was first performed. The mean of the control experiments was 949 with standard deviation 58. However, the frequency histogram and other parameters of descriptive statistics indicated that the probability distribution of NPFC was asymmetrical and could be represented as log–normal with the logarithmic mean of NPFC/test chamber as 904 and the 95% confidence interval 806 to 1016. (This means that individual series of controls fell with 95% probability into this interval). In contrast, the probability distribution of the number of surviving cells at the end of experiments (viability) was found to be close to normal distribution, with the mean value 3.18×10^6 and the 95% confidence interval from 2.86×10^6 to 3.51×10^6. To evaluate how the steroids did affect the NPFC, the modified Dunn's test was applied thus. At first, the null hypothesis about the NPFC mean of control and experiment ($\mu_e = \mu_c$) was tested. Secondly, when the test failed, the alternative hypothesis for significance of steroid effect on primary immune response ($\mu_e < \mu_c$ or $\mu_e > \mu_c$) was examined. The comparison of both means was performed according to the formula:

$$|\ln X_E - \ln X_C| > t_{1-\alpha/2} s_p [(n_e + n_c)/n_e n_c]^{1/2}$$

where X_E and X_C were means of NPFC of experiments and controls, respectively. The value of s_p was calculated according to the formula $s_p^2 = \{[(n_e-1)s_e^2 + (n_c-1)s_c^2]/(n_e + n_e - 2)\}$. The corresponding degree of freedom was $\nu = n_e + n_c - 2$, the other parameters used were $n_c = 28$, $s_c = 0.0988$ and $X_C = 904$.

Table 1
The effect of various doses of dexamethasone, DHEA and both (7α- and 7β) isomers of 7-OH-DHEA on the number of plaque forming cells (NPFC) in cultured murine spleen lymphocytes. Results are expressed as % of the mean value of NPFC from 28 control experiments. The data represent the mean values from at lest 3 values for each concentration of each steroid, i.e. altogether 28 experiments. Doses of added steroid are given in mg/L; the lowest dose 0.01 mg/L corresponds to the concentrations 25, 34.7 and 32.9 nM for dexamethasone, DHEA and 7-OH-DHEA, respectively. Asterisks show significance of the effect of tested compound related to controls at 95% probability level

	The effect of tested compound on the NPFC (in % of controls)			
steroid/dose (μg/mL)	0.01	0.1	1	10
DHEA	47.8*	48.7*	83.1	13.6*
7α-OH-DHEA	87.7	20.5*	42.1*	51.1*
7β-OH-DHEA	23.3*	22.2*	40.1*	56.1*
Dexamethasone	3.3*	3.4*	5.1*	16.5*

Table 2
The effect of various doses of dexamethasone, DHEA and both (7α- and 7β) isomers of 7-OH-DHEA on the cell viability in cultured murine spleen lymphocytes. Results are expressed as % of surviving cells at the end of the experiment related to the mean value of control experiments. The data represent the mean values from at lest 3 values for each concentration of each steroid, i.e. altogether 28 experiments. The doses of tested steroids are the same as in Table 1. Asterisks show significance of the effect of tested compound related to controls at 95% probability level

	The effect of tested compound on the cell viability as % of surviving cells at the end of the experiment related to the mean value of control experiments			
steroid/dose (μg/mL)	0.01	0.1	1	10
DHEA	64.9	103	126	77.6
7α-OH-DHEA	92.2	90.7	88.6	81.2
7β-OH-DHEA	91.5	103	101	97.7
Dexamethasone	33.5*	37.7	42.7	42.9*

3. Results and discussion

The effect of increasing doses of dexamethasone, DHEA and of both isomers of 7-OH-DHEA on the NPFC is shown in Table 1, and the effect of these steroids on cell viability at the end of experiments is shown in Table 2. As expected, dexamethasone suppressed the primary response even at the lowest concentration (0.01 μg/ml, i.e. 25.5 nM) and it had also a significantly toxic effect on cell viability in contrast to other steroid tested. The effect of other steroids on the NPFC differed considerably. While DHEA and its 7α-hydroxyderivative decreased the NPFC irrespectively to the dose, the decrease of the NPFC caused by 7β-OH-DHEA was decreased along with increasing dose.

For a better understanding of this effect, the data from all the experiments testing the effect of the steroids were plotted against the logarithm of their concentrations (since the log of zero is not defined, the 100 times lower concentrations than that of the lowest actual steroid concentration were attributed to control experiments), as shown on Fig. 1. As mentioned above, DHEA and its 7α-hydroxylated metabolite decreased slightly the primary immune response and the lines representing the upper and lower borderline were close to parallel. The inhibitory effect of 7β-OH-DHEA exhibited a considerably higher variance at higher concentrations, which may be explained by a diverse effect on various cell populations.

Therefore, the simultaneous effect of the latter steroid and dexamethasone on the NPFC as well as on cell viability was tested. The concentration of dexamethasone was kept constant, chosen as such to cause already a significant decrease of the NPFC (see Table

Table 3
Simultaneous effect of dexamethasone and of 7β-OH-DHEA on the number of plaque forming cells (NPFC) and their viability at the end of the experiment in cultured murine spleen lymphocytes. The data are expressed as % of the effect of the dexamethasone alone (0.1 μg/ml, i.e. 255 nM, mean from 9 experiments). Each value represents the mean from three parallel experiments. Asterisks show significance of the effect of tested compound related to that of dexamethasone alone at 95% probability level (% of the effect of dexamethasone)

Dose of 7β-OH-DHEA	NPFC (%)	Viability (%)
0.1 μg/ml (347 nM)	122.8	94.4
1.0 μg/ml (3.47 μM)	155.5*	116.7
10 μg/ml (34.7 μM)	150.8*	116.7

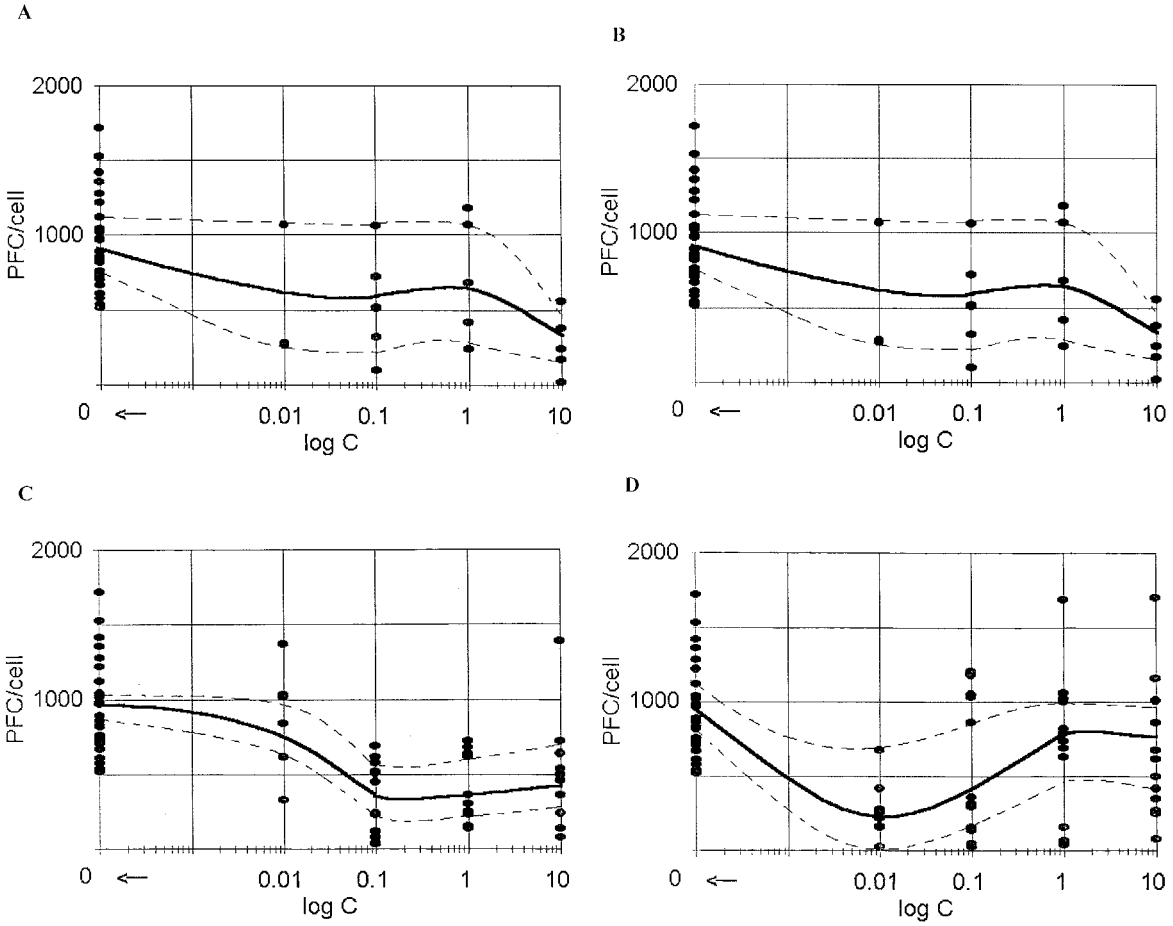

Fig. 1. The effect of various doses of dexamethasone, DHEA and both (7α- and 7β) isomers of 7-OH-DHEA on the number of plaque forming cells (NPFC) in cultured murine spleen lymphocytes. Results are expressed as % of the mean value of NPFC from 28 parallel control experiments in dependence on the logarithm of the respective steroid concentration. Black circles: experimental data, bold line: mean, dashed lines: estimation of limits of the mean value on 95% level of significance using z-test. (A) DHEA, (B) dexamethasone, (C) 7α-OH-DHEA, (D) 7β-OH-DHEA.

1), i.e. 0.1 μg/ml (255 nM), while 3 concentrations of 7β-OH-DHEA were tested. The effect of dexamethasone alone (the mean from 9 experiments) was taken as a reference value to which the simultaneous effect of 7β-OH-DHEA was related. The results are summarised in Table 3. Each value represents the mean from 3 parallel experiments. It may be seen that 7β-OH-DHEA counteracted the effect of dexamethasone on NPFC even at the same concentration as dexamethasone but it did not counteracted the toxic effect of dexamethasone on cell viability.

The finding that 7β-OH-DHEA is able to counteract at least partially the immunosuppressive effect of dexamethasone is in agreement with the recent results of others [12,13], who demonstrated that another 7β-hydroxylated metabolite of DHEA, namely 5-androstene-3β,7β,17β-triol but not DHEA itself acted in a similar way.

Acknowledgements

This study was supported by the Grants No. 0360-3 of the Grant Agency of the Czech Republic, and No. 4205-3 of the Internal Grant Agency of the Czech Ministry of Health.

References

[1] E.E. Baulieu, Dehydroepiandrosterone (DHEA): a fountain of youth, J. Clin. Endocr. Metab. 81 (1996) 3147–3151.
[2] R.R. Watson, A. Huls, M. Araghinikuam, S. Chung, Dehydroepiandrosterone and diseases of aging, Drugs Aging 9 (1996) 274–291.
[3] B. Araneo, T. Dowell, M.L. Woods, R.A. Daynes, M. Judd, T. Evans, DHEAS as an effective vaccine adjuvant in elderly humans, in: F.L. Bellino, R.A, Daynes, P.J. Hornsby, D.H. Lavrin, J.E. Nestler (Eds.), Dehydroepiandrosterone (DHEA)

and aging, Ann. N. Y. Acad. Sci. 774, New York 1995, pp. 232–247.
[4] N.J. Olsen, W.J. Kovacs, Gonadal steroids and immunity, Endocrin. Rev. 17 (1996) 369–384.
[5] M. Kalimi, Y. Shafagoj, R. Loria, D. Padgett, W. Regelson, Anti-glucocorticoid effects of dehydroepiandrosterone (DHEA), Mol. Cell. Biochem. 131 (1994) 99–104.
[6] B. Araneo, R. Daynes, Dehydroepiandrosterone functions as more than an antiglucocorticoid in preserving immunocompetence after thermal injury, Endocrinology 136 (1995) 393–401.
[7] P.F. Mohan, M.P. Cleary, Studies on nuclear binding of dehydroepiandrosterone in hepatocytes, Steroids 57 (1992) 244–247.
[8] R. Hampl, R. Morfin, L. Stárka, 7-Hydroxylated derivatives of dehydroepiandrosterone: what are they good for ?, Endocr. Regul. 31 (1997) 211–218.
[9] Y. Akwa, N. Sananes, M. Gouezou, P. Robel, E.E. Baulieu, C. Goascogne, Astrocytes and neurosteroids: metabolism of pregnenolone and dehydroepiandrosterone. Regulation by cell density, J. Cell. Biol. 121 (1993) 135–143.
[10] M.W. Khalil, B. Strutt, D. Vachon, D.W. Killinger, Effect of dexamethasone and cytochrome P450 inhibitors on the formation of 7α-hydroxydehydroepiandrosterone by human adipose stromal cells, J. Steroid Biochem. Mol. Biol. 48 (1994) 545–552.
[11] R. Morfin, personal communication.
[12] R.M. Loria, Antiglucocorticoid function of androstenetriol, Psychoneuroendocrinology 22 (Suppl. 1) (1997) S103–S108.
[13] R.M. Loria, D.A. Padgett, Control of the immune response by DHEA and its metabolites, Rinsho Byori 46 (1998) 505–517.
[14] L. Starka, M. Hill, R. Hampl, M-I. Malewiak, A. Benalycherif, R. Morfin, J. Kolena, S. Scsukova, Studies on the mechanism of antiglucocorticoid action of 7α-hydroxydehydroepiandrosterone, Collect. Czech. Chem. Commun. 63 (1998) 1683–1698.
[15] L. Stárka, Reaktion der Steroide mit tert-Butylperbenzoat I. Über die 7-Acyloxylierung Δ^5-unsättiger Steroide, Collect. Czech. Chem. Commun. 26 (1962) 2452–2456.
[16] J. Šterzl, J. Milerová, L. Macková, J. Trávníček, Induction and recall of the secondary immune response entirely in tissue culture, Adv. Exp. Med. Biol. 371A (1995) 1–8.
[17] J. Šterzl, R. Mandel, Estimation of the inductive phase of antibody formation by plaque technique, Folia Microbiol. 9 (1964) 173–175.