METABOLITIC ACTIVITY OF 11-DEOXYCORTICOSTERONE AND PREDNISOLONE IN THE ALGA *CHLORELLA VULGARIS* BEIJERINCK

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ABSTRACT

The influence of optimal concentrations 5×10^{-6}M - 10^{-6}M of 11-deoxycorticosterone (mineralocorticoid) and prednisolone (glucocorticoid) on the growth (fresh and dry weight) and content of soluble proteins, reducing sugars and nucleic acids in the green alga *Chlorella vulgaris* (Chlorophyceae). Both corticosteroids at concentration 5×10^{-6}M were most strongly active metabolically between the 5th-15th day of the cultivation and this probably was caused by their chemical biotransformation. The applied corticosteroids induced the strongest stimulative effect on the content of soluble proteins in the range of 167-196% and reducing sugars (233-275%) when compared to the control (100%). Prednisolone showed lower stimulative activity on the content of proteins. But 11-deoxycorticosterone showed weaker stimulation of on the content of sugar. Both of the corticosteroids showed a stimulating or inhibitory influence upon the content of nucleic acids in *C. vulgaris* cells without regard to the concentration.

KEY WORDS: *Chlorella vulgaris*, deoxycorticosterone, prednisolone, proteins, nucleic acids, reducing sugars.

INTRODUCTION

In many species of vascular plants, algae and fungi, different steroid hormones have been isolated so far from many groups: estrogens, androgens, brassinosteroids, corticosteroids and ecdysteroids (Czerpak 1993; Bajguz and Czerpak 1995; Lafont 1997). Typical corticosteroids (gluco- and mineralocorticoids) and their analogues, have been isolated from many species of grasses and cereals (Graminae), as well as from doubledeciduous plants mostly belonging to the families: Compositae, Cruciferae, Cucurbitaceae, Labiatae, Papilionaceae and Scrophulariaceae, mainly of genus *Digitalis*. The plant species of which are the richest source of corticosteroids include: bean, wheat, rice, lentil, liquorice, *Digitalis purpurea* and fungi from the genera *Rhizopus* and *Cuninghamella*. Such corticosteroids as: cortisone, cortisole, corticosteroid, 11-dehydrocortisone, 18-hydroxycorticosteron, aldosterone, 11-deoxycortisone and 11-deoxycorticosterone were most often reported to be present in a variety of plants (Skarżyński 1933; Grünwald 1975; Heftmann 1975; Geuns 1978; Sláma 1980; Cutler et al. 1991; Duperon et al. 1992).

The physiological and metabolitic activity of corticosteroids in animals is conditioned by the presence of hydroxyl or ketone groups with carbon atoms in the positions: C-11, C-17 and C-21. Hydroxylation of carbon at C-21 is indispensable for the biological activity of gluco- and mineralocorticoids in mammals, whereas an additional oxidation process of carbon atoms at C-11 and C-17 to hydroxyl or ketone groups strengthens their glucocorticosteroid properties and weakens the mineralocorticosteroid ones. In plants, the most biologically glucocorticoids to stimulate seed germination, growth, development and physiological-metabolic processes of young plants are those ones which include an oxygen atom at C-11 in the form of hydroxyl or ketone groups. However, mineralocorticoids deprived of oxygen at C-11 produce mostly an adverse effect in comparison to glucocorticoids because they delay seed germination, vegetative growth of plants and some biochemical processes connected with it (Geuns 1974, 1977, 1983; Johnson and Baxter 1978).

It appeared that animal steroid hormones, when introduced into plants, can undergo a specific biochemical modification called biotransformation. In some vascular plants and algae from the group of: Phaeophyta, Euglenophyta, Rhodophyta and Chlorophyta, systems were present oxygenating carbon atoms at C-11 and C-17 located in the corticosteroid skeleton with the participation of cytochrome P-450, which in mammals had been detected much earlier in the mitochondria of kidney cortex cells. It is known from the hithero research that the greatest biological activity in plants is shown by these animal exogenous steroids or their chemical synthetic analogues, which possess a ketone group at C-3, a hydroxyl group at C-11 and C-17, and carbonyl group one at C-20 (Geuns 1978; Hewitt et al. 1980; Guan and Roddick 1988; Jones and Roddick 1988; Bajguz and Czerpak 1996, 1998).

The biosynthetic pathway of phytosteroids in plants follows a pattern similar to that in animals up to the moment of producing squalen (30-carbon aliphatic hydrocarbon), which in oxygen conditions undergoes cyclisation into sterols. The main synthetic sterols include: cholesterol, cycloartenole, lanosterole, pregnenolone and ergosterole, which are the most often direct substrates in the biosynthesis of different steroid structures with a hormonal function, or with another one

regulating physiological-metabolic processes of organisms. In the biosynthesis of plant corticoids, pregnenolone is converted into progesterone and 11-hydroxyprogesterone, which, as a result of futher hydroxylation or oxygenation changes, most often generate: corticosterone, 11-deoxycorticosterone, cortisole, 11-deoxycortisole, aldosterone and many others (Grünwald 1975; Heftmann 1978; Geuns 1983; Harrison 1985; Pollio et al. 1994).

In auto- and heterotrophic cells, steroids hormones can undergo mutual chemical transformations, such as: hydroxy-, oxy-, hydro-, and dehydro-, by the addition or separation of oxygen or hydrogen atoms within the steroid skeleton and aliphatic chain. The steroids can easily form ester and glycoside compounds called "conjugate compounds", which, depending on physiological and metabolic needs, are hydrolised into biologically active free forms (Sláma 1980; Geuns 1983; Mahato and Majumdar 1993; Pollio et al. 1994).

According to the above-mentioned facts, the authors' research was focused on comparative analyses of metabolitic activity on the grounds of content changes of water-soluble proteins, reducing sugars and nucleic acids in *Chlorella vulgaris* under the influence of optimal concentrations of 11-deoxycorticosterone – a typical mineralocorticoid, and prednisolone (a synthetic analogue of cortisole) belonging to glucocorticoids.

MATERIALS AND METHODS

General methodological principles

The experiments were carried out on fresh homogenous culture of the alga *Chlorella vulgaris* Beijerick (Chlorophyceae). The algae were grown for 20 days in 500 cm^3 conical flasks with bacteriological stoppers, containing 250 cm^3 of the modified Knop medium, under controlled conditions at 25 (± 1)°C. Illumination was supplied 16h photoperiod (8h dark period) by a bank of fluorescent lights yielding a photon flux of 50 μmol/ $m^{-2} \times s^{-1}$ at the surface of the tubes (Stewart 1974). Permanent synchronouns growth was established according to the method of Pirson and Lorenzen (1966), in the conditions developed by Sayegh and Greppin (1973). The pH of the medium was adjusted to 6.8 with 1N NaOH. The algae were grown in the medium supplemented with the following additional corticosteroids: deoxycorticosterone and prednisolone (Polfa, Poland), in optimal molar concentrations 5×10^{-6} and 10^{-6} M. The steroids were dissolved in 50% ethanol, and 10 μl of the solution was applied to the cultures. Corticosteroids were not added to the control algal culture but it was treated with 10 μl of 50% ethanol.

Determination of the content of fresh and dry weight

A method of common use in the laboratory practice, was applieded after Hallegraef (1977), Lee and Low (1993). To determine the fresh and dry weight, two 10 ml algal cultures were filtered through preweighed glass microfiber filters (Whatman, Maidstone, UK) washed with distilled water to remove non-biological materials, such as mineral salts precipitates, dried overnight at 105°C for a few hours to a constant weight. The fresh and dry weight was expessed in g on dm^3 of the algal culture.

Determination of the content of water-soluble proteins, nucleic acids and reducing sugars

The total content of these biological parameters was determined as g% of *Chlorella vulgaris* dry weight. The fraction of water-soluble proteins was analysed spectrophotometrically using biuretic reaction after homogenisation of the algal cells and their water extraction.

A similar preparation procedure was employed for spectrophotometic determination of reducing sugars, according to Samogyi-Nelson's (Hodge and Hofreiter 1962) method. The total content of nucleic acids (DNA + RNA) was determined spectrophotometrically using the orcine reagent, as described by Ostrowski and Filipowicz (1981).

A UV/Vis spectrophotometer (DU-640, Beckman, USA) was used for all the measurments.

RESULTS

Both applied corticosteroids show the greatest biological activity in a unicellular alga *Chlorella vulgaris* between 5th and 15th day of cultivation, where deoxycorticosterone was more active biochemically than prednisolone. The results of the research presenting the contents of the analysed biochemical parameter in *Chlorella vulgaris* under the influence of optimal concentrations 5×10^{-6} M and 10^{-6} M 11-deoxycorticosterone and prednisolone are included in Table 1. The percentage contents of biochemical parameters, with their maximum stimulatory effect under the influence of the analysed corticosteroids in comparison to the control cultivation of algae (100%), are presented graphically in Fig. 1.

The growth of algae under the influence of applied corticosteroids was most intensively stimulated between the 5th and 10th day of cultivation. The content of fresh weight reached its maximum on the 5th day of algae cultivation, reaching 239% under the influence of 11-deoxycorticosterone, and 206% – under the influence of prednisolone. However, the dry weight was the highest on the 10th day of algae cultivation, reaching the values of: 134% – under the influence of 11-deoxycorticosterone, and 110% – under the influence of prednisolone, when compared to the control (100%).

The content of peptides and water soluble proteins was most intensively stimulated on the 15th day of algae cultivation – in the range of 267% under the influence of 11-deoxycorticosterone, and 296% – when affected by prednisolone, which in this case appeared to be more active. Moreover, the total content of nucleic acids (DNA and RNA) was slightly inhibited from the 10th day of *Chlorella vulgaris* cultivation, but, starting with the 15th day of the experiment, it was minimally stimulated, reaching the values: 103% – under the influence of prednisolone, and 106% – under the influence of 11-deoxycorticosterone when compared to the control.

The greatest stimulation throughout the whole 20 day period of *Chlorella vulgaris* cultivation under the influence of both applied corticosteroids, was observed in the case of the content of reducing sugars. The maximum content of the above-mentioned sugars reached the value of 333% on the 5th day of cultivation under the influence of prednisolone whereas, under the influence of 11-deoxycorticosterone, it rose up to 375% on the 10th day of cultivation when compared to the control.

The obtained results indicate that prednisolone as a typical synthetic glucocorticoid intensively stimulates the analysed biochemical parameters from the 5th day of cultivation, with the exception of nucleic acids. In turn, 11-deoxycorticosterone showed the greatest stimulation on the biochemical parameters of *Chlorella vulgaris* from the 10th day of cultivation.

TABLE 1. Content of the analyzed biochemical parameters at optimal concentration of corticosteroids in *Chlorella vulgaris* cells.

Time of culture	Optimal molar concentration	11-Deoxycorticosterone					Prednisolone				
		Fresh weight	Dry weight	Water-soluble proteins	Reducing sugars	Nucleic acids	Fresh weight	Dry weight	Water-soluble proteins	Reducing sugars	Nucleic acids
(days)	(M)	(g/dm^3)	(g/dm^3)	(g% of the dry wt.)	(g% of the dry wt.)	(g% of the dry wt.)	(g/dm^3)	(g/dm^3)	(g% of the dry wt.)	(g% of the dry wt.)	(g% of the dry wt.)
5	5×10^{-6}	12.92	1.65	2.01	0.11	4.19	11.13	1.38	2.56	0.10	4.02
	10^{-6}	10.02	1.54	1.87	0.09	4.01	9.61	1.33	1.54	0.08	3.98
	control	5.41	1.30	1.25	0.03	4.22	5.41	1.30	1.25	0.03	4.22
10	5×10^{-6}	13.62	1.80	3.25	0.30	4.36	12.48	1.47	4.48	0.21	4.24
	10^{-6}	11.38	1.57	3.03	0.17	4.24	9.88	1.38	2.05	0.15	4.19
	control	6.63	1.34	1.67	0.08	4.33	6.63	1.34	1.67	0.08	4.33
15	5×10^{-6}	14.71	1.98	5.01	0.36	4.68	14.42	1.58	5.56	0.30	4.52
	10^{-6}	12.21	1.60	3.60	0.24	4.35	10.23	1.53	2.88	0.20	4.33
	control	7.33	1.48	1.88	0.11	4.40	7.33	1.48	1.88	0.11	4.40
20	5×10^{-6}	14.86	2.05	5.36	0.46	4.46	12.81	1.60	6.36	0.37	4.41
	10^{-6}	12.55	1.69	4.14	0.30	4.30	10.64	1.67	3.15	0.24	4.22
	control	7.36	1.54	2.25	0.13	4.45	7.36	1.54	2.25	0.13	4.45

Mean values from twenty analyses; S.E. <5%

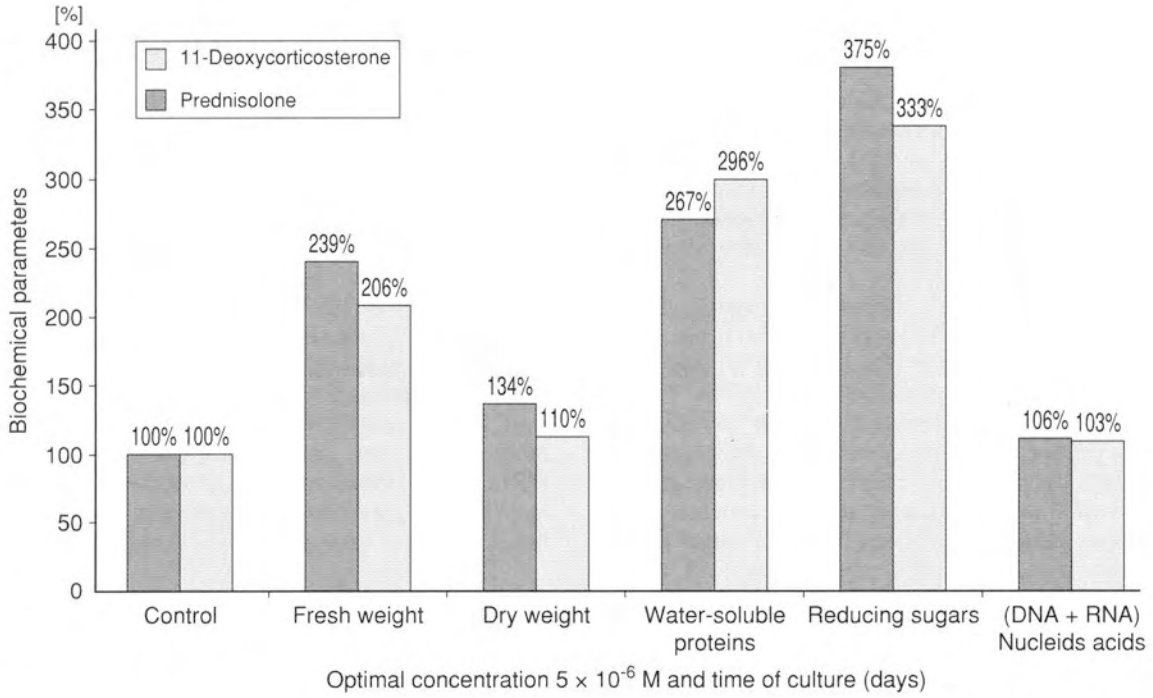

Fig. 1. Maximal stimulation of the biochemical parameters at the optimal comcetration 5×10^{-6}M 11-deoxycorticosterone and prednisolone between 5-15 day cultures in *Chlorella vulgaris* cells. Mean values (n=20).

DISCUSSION

It results from hithero existing scanty empirical research conducted mainly on vascular plants (Caspi et al. 1968; Sláma 1980; Czerpak 1993) that animal corticoids, particularly glucocorticoids and some of their analogues, show a considerable physiological-metabolic activity in plants. The research done on germinating seeds and young plants of bean (*Phaseolus vulgaris*) while using maximum concentration of glucocorticoids: cortisole, corticosterone and prednisolone, only after two or three days showed stimulative effect on the growth of overground sprouts and roots, particularly the later ones, in the range from 20 to over 50%. Stimultaneousy, not only does the content of biomass increase under the influence of applied corticosteroids but also the content of dry weight, proteins and ribonucleic acids, particularly in the developing embryos and young plants at the initial stage of autotrophism. However, mineralocorticoids, e.g. aldosterone and 11-deoxycorticosterone and their methylene analogues showed antagonistic influence to typical glucocorticoids because they in-

hibited germination of seeds and generative growth of plants. On the other hand they had a stimulative effect on the growth of roots, particularly the lateral and adventitious ones, and the content of proteins and RNA (Geuns 1974, 1977, 1983; Loeys and Geuns 1978).

Studies conducted on the unicellular alga *Chlorella vulgaris* under the influence of optimal concentration of 5×10^{-6} M of 11-deoxycorticosterone and prednisolone – showed stimulating activity and there was found an appreciable stimulation of the growth of algal cells, which was evidenced by the increase of biomass from 106 to 139%, and dry weight from 10 to 34% in reference to the control cultivation. Both the applied corticoids proved the highest stimulation of the content growth of the fraction of water-soluble proteins in the range of 167-196%, and of reducing sugars from 233 to 275%. In turn, the total content of nucleic acids (DNA + RNA) was slightly inhibited only after 5 days and after a lapse of 15 days of algae cultivation it was stimulated in the range of 3-6% as compared to the control. The two applied corticoids showed the highest stimulative activity in relation to the analysed biochemical parameters in *Chlorella vulgaris* between the 5th and 15th day of cultivation. 11-deoxycorticosterone revealed a little higher stimulation, exept water-soluble proteins, where prednisolone was biologically more active.

Partly similar research results were obtained by Bajguz and Czerpak (1996) during the experiments on *Chlorella vulgaris* with the use of estradiol (a steroidal female hormon), which demonstrated the greatest stimulative activity in very small concentrations in the range of 10^{-11}-10^{-12} M, also between the 5th and 15th day of cultivation. Estradiol was found to have influenced the greatest content stimulation of: chlorophylls *a* and *b* (in the range of 26-35%), carotenoids – on the average 75%, water-soluble proteins – 15-20% and reducing sugars 23-25%, in reference to the control sample. However, the concentrations of estradiol higher than 10^{-6} M caused gradual inhibition of algal growth and the total amount of the analysed biochemical parameters.

In their experiments with bean (*Phaseolus vulgaris*), pumpkin (*Cucurbita pepo*), chicory (*Cichorium intybus*), pea seedlings (*Pisum sativum*), pine seeds (*Pinus silvestris*) and other species of vascular plants, Kopcewicz (1969, 1970), Kopcewicz and Rogozińska (1972) demonstrated that steroidal female hormones: estrone, estradiol and estriol in the concentrations of 0.1-0.001 µg per plant stimulated the plant growth and the process of flower formation, anabolic processes and the biosynthesis of auxins and gibberellins in the range of 30-40%, while they reduced significantly the level of ABA. Among the applied estrogens: estradiol and estriol displayed a much greater stimulative activity in comparison to estron and other homologous compounds. Also Geuns (1978) research on germinating seeds and young plants of pea (*Pisum sativum*) and carrot (*Daucus carota*) shows that estriol used in concentrations of 25-100 mg/l intensively stimulated the growth and the content of chlorophylls.

In other experiments, conducted on *Phaseolus aureus*, Geuns (1974, 1977, 1983), Loeys and Geuns (1978), studying the biological activity of over forty different natural and synthetic chemical analogues of corticoids, proved the strongest stimulative effect on the growth of hypocotyl and roots, particularly the lateral ones, under the influence of corticosterone, cortisone, 21-deoxycortisone, 21-deoxycortisole, dihydrocortisone, 3-hydroxydihydrocortisone, 11-β-hydroxyprogesterone (a direct precursor of corticosterone), β-cortolone, prednisolone and dexametasone. However, typical mineralocorticoids such as: aldosterone, 11-deoxycortisol and others deprived of oxygen at C-11, hampered the formation and growth of the roots, and the elongation of hypocotyls in developing embryos of sprouting bean seeds. It appeared that a hydroxyl or ketone group at C-11 is necessary in order to stimulate the growth of hypocotyls and roots, especially the lateral ones, under the influence of typical animal glucocorticoids. However, the absence of oxygen at C-21 or hydrogenation of a double bond in the skleroidal skeleton slightly reduces their biological activity. Corticosteroids containing a hydroxy group at C-11 in the isomeric β form show the greatest stimulatory activity while their α form is weakly active.

From Geuns's (1983) reports on bean, and also from Abul-Haija and Qiana's (1986) and Mahato and Majumdara's (1993) works on many microorganisms belonging to: bacteria, algae and fungi, it is know that they possess appropriate enzymes, mostly C-11, C-17 and C-21 hydroxylases or oxydoreductases, which produce biochemical transformation of typical endogenous and exogenous animal corticosteroids. It was proved empirically that many taxonomically different groups of algae, e.g. *Anabaena*, *Anacystis*, *Ankistrodesmus*, *Chlorococcum*, *Characium*, *Chlorogloea*, *Oscillatorium*, *Phormidium*, *Scenedesmus*, probably *Chlorella* and the other algae, possess great biotransformation abilities of exogenous steroids. For example, after the introduction of 4-androstendione-3.17 into algal cells under the influence of specific oxydoreductase, its ketone group at C-17 was reduced to a hydroxyl group, thus producing testosterone and its hydroxyl homologues, such as 14-hydroxytestosterone.

It is well known that β-hydroxylation at C-11 can occur in mammals mitochondria of adrenal cortex in the presence of P-450 cytochrome, which leads, among others, to the conversion of mineralocorticoids into glucocorticoids (Jacob et al. 1975; Johnson and Baxter 1978; Sláma 1980). All this points out the fact that plants, among them also algae, most probably possess specific enzymes which catalyse the oxyreduction of proper carbon atoms in the molecules of exo- and endogenous steroids (Abul Haij and Qian 1986; Cutler et al. 1991; Mahato and Majumdar 1993; Pollio et al. 1994).

Therefore, it should be supposed that 11-deoxycorticosterone, exogenously penetrating *Chlorella vulgaris* cells, in a short time underwent biotransformation to some glucocorticoid which stimulated the growth and content of water-soluble proteins and reducing sugars in the algae. Stimulative effect of 11-deoxycorticosterone on algal cells, in comparison to synthetic glucocorticoid – prednisolone, was slightly delayed, probably because of its biotransformation.

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METABOLICZNA AKTYWNOŚĆ 11-DEOKSYKORTYKOSTERONU I PREDNIZOLONU W GLONIE *CHLORELLA VULGARIS* BEIJERINCK

STRESZCZENIE

Badano wpływ optymalnych stężeń 5×10^{-6}M - 10^{-6}M 11-deoksykortykosteronu (mineralokortykoid) i prednizolonu (glukokortykoid) na tempo wzrostu (zawartość świeżej i suchej masy) i zmiany w zawartości białek rozpuszczalnych, cukrów redukujących i kwasów nukleinowych (DNA + RNA) w jednokomórkowej *Chlorella vulgaris* (Chlorophyceae). Badania wykazały, że oba kortykosteroidy są najbardziej aktywne metabolicznie w stężeniu 5×10^{-6}M między 5 a 15 dniem hodowli *Chlorella vulgaris*. Najprawdopodobniej jest to spowodowane ich biochemiczną transformacją w komórkach glonów. Oba kortykosteroidy powodują znaczny przyrost zawartości bialek rozpuszczalnych w granicach 167-196% i cukrów redukujacych w zakresie 233-275% w stosunku do hodowli kontrolnej (100%). Nieco większą aktywność biochemiczną w stymulacji zawartości białek wykazuje prednizolon, zaś cukrów 11-deoksykortykosteron. W zależności od wielkości stosowanych stężeń oba stosowane kortykoidy wykazują albo nieznaczną stymulację w granicach 3-6% lub obniżenie o kilka procent ogólnej puli kwasów nukleinowych w glonie *Chlorella vulgaris*.

SŁOWA KLUCZOWE: *Chlorella vulgaris*, deoksykortykosteron, prednizolon, białka, kwasy nukleinowe, cukry redukujące.