Vol. 119, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 29, 1984 Pages 401-407

MODULATION BY DEXAMETHASONE OF THE FATTY ACYL-CoA DESATURASE SYSTEM IN TETRAHYMENA MICROSOMES

Shigenobu Umeki and Yoshinori Nozawa

Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi-40, Gifu, Japan

Received January 17, 1984

Dexamethasone produced an increased activity of stearoyl-CoA desaturase through the enhancement of Δ^9-terminal component activity, and a corresponding decrease of oleoyl-CoA desaturase activity via the reduced activity of Δ^{12}-terminal component in Tetrahymena microsomes. However, the content of cytochrome b_5 as well as the activities of NAD(P)H-cytochrome c and NADH-ferricyanide reductases showed no significant changes by dexamethasone. Additionally, dexamethasone evoked a 3.5-fold increase of intracellular cyclic AMP content 2 hr after administration. These results suggest that dexamethasone may modulate microsomal fatty acyl-CoA desaturase system in Tetrahymena by increasing intracellular cyclic AMP content.

INTRODUCTION: Fatty acyl-CoA desaturase (EC 1.14.99.5) plays a crucial role in control mechanisms by which a wide range of organisms achieves various adaptive modification of fatty acid composition in membrane lipids and preserves an adequate membrane fluidity to maintain a favorable membrane function in response to its environmental changes, such as temperature acclimation [1-4], nutritional changes [5, 6], anaerobiosis [7] and hormone exposure [8-11]. In particular, the reports on hormonal regulation of this enzyme activity have recently been increasing. It is well known that insulin and thyroid hormone are involved in the control of microsomal fatty acyl-CoA desaturase system [8-10]. We have also obtained evidence that β-adrenergic agonists change the fatty acyl-CoA desaturase activities in microsomes from a unicellular eukaryote, Tetrahymena pyriformis [Umeki and Nozawa, manuscript in preparation]. It has been demonstrated, in addition, that glucocorticoids alter Δ^9-, Δ^6- and Δ^5-desaturase activities [11].

In the present study, we have investigated the influence of dexamethasone on the fatty acyl-CoA desaturase system in Tetrahymena microsomes, and proposed a hypothesis for its possible mechanism for the regulation of this system by dexamethasone.

0006-291X/84 $1.50

MATERIALS AND METHODS:

Materials: The following chemicals were obtained from commercial sources: [1-^{14}C]stearoyl-CoA (59.6 mCi/mmol) and [1-^{14}C]oleoyl-CoA (59.7 mCi/mmol) (New England Nuclear, Boston, MA); cyclic AMP radioimmunoassay kit (Yamasa Shoyu Co. Ltd., Tokyo); stearoyl-CoA and oleoyl-CoA (P-L Biochemicals Inc., Milwaukee, WI); NADH(NADPH), horse heart cytochrome c and dexamethasone (Sigma Chemical Co., St. Louis, MO); potassium ferricyanide (Kishida Chemical Co. Ltd., Osaka). Other chemicals were of the highest purity available from commercial sources.

Cell growth and isolation of microsomes: A thermotolerant strain NT-1 of Tetrahymena pyriformis was grown at 39°C in 500 ml Erlenmeyer flasks containing 200 ml of an enriched proteose-peptone medium (2 % proteose-peptone, 0.2 % yeast extract and 90 μM EDTA-Fe^{3+} complex) without glucose [2]. When the density of cells reached 4.0 x 10^4 cells/ml, 10 μM of dexamethasone was added to the medium and culture was further incubated for the indicated periods at 39°C. At different intervals, cells were withdrawn and homogenized in 5 volumes of phosphate buffer (0.2 M K_2HPO_4/0.2 M KH_2PO_4, 3 mM EDTA and 0.1 M NaCl, pH 7.4) with a hand glass homogenizer (A.H. Thomas Co., USA), and microsomal fractions were prepared from homogenates, as previously described [12]. The microsomes obtained were washed once by suspending in 50-100 ml of fresh 0.1 M potassium phosphate buffer (pH 7.4). Washed microsomes were immediately frozen at -90°C before use. Protein was determined according to the method of Lowry et al. [13], using bovine serum albumin as standard.

Enzyme assays: For the assay of fatty acyl-CoA desaturases, typical reaction mixtures contained 20 nmol of [1-^{14}C]stearoyl-CoA (1 Ci/mol) or [1-^{14}C]oleoyl-CoA (1 Ci/mol) as substrate, 50 nmol of NADH as an electron donor, suitable amounts of microsomes (0.10 mg for stearoyl-CoA desaturase assay and 0.40 mg for oleoyl-CoA desaturase assay), and 0.1 M potassium phosphate buffer (pH 7.2) to a final volume of 0.5 ml. Each sample was preincubated for 1 min at 39°C prior to the addition of microsomes to initiate the reaction. After incubation for 3 min at 39°C the reaction was stopped by the addition of 0.5 ml of 10 % KOH in methanol. Analysis of reaction products was performed according to the method of Oshino et al. [14] with a slight modification [15]. Labeled fatty acid methyl esters separated by a JEOL Model JGC-1100 gas chromatograph were trapped by insertion of glass tubes (5 x 150 mm) with open ends, and eluted from the tubes with toluene into scintillation vials. The radioactivity was determined with a Beckman scintillation counter.

The NAD(P)H-cytochrome c and NADH-ferricyanide reductase activities were determined using 20 nmol of cytochrome c or 500 nmol of potassium ferricyanide, 100 nmol of NADH or NADPH, 20-100 μg of microsomes, and 0.1 M potassium phosphate buffer (pH 7.4) in a total volume of 1.0 ml at 34°C [3, 7]. The activities were calculated using respective extinction coefficients of 19.6 $mM^{-1} \cdot cm^{-1}$ [16] and 1.02 $mM^{-1} \cdot cm^{-1}$ [17].

Cytochrome b_5 was reduced by the addition of 100 nmol of NADH and the content of cytochrome b_5 was determined by measuring the reduced minus oxidized difference spectrum, taking the extinction difference of the cytochrome between 425 and 410 nm as 216 $mM^{-1} \cdot cm^{-1}$ [18].

Terminal component activity was assayed by measuring spectrophotometrically the palmitoyl-CoA-stimulated reoxidation rate of NADH-reduced cytochrome b_5 by monitoring the change in absorbance difference between 425 and 410 nm. The sample cuvette contained microsomes with 0.2 nmol of cytochrome b_5, 200 nmol of fresh Na_2S to inhibit mitochondrial cytochrome oxidase, and 0.1 M Tris-HCl buffer (pH 7.2) in a final volume of 3.0 ml. The rate of reoxidation of cytochrome b_5 reduced by 2 nmol of NADH at 34°C was calculated using 216 $mM^{-1} \cdot cm^{-1}$ as the extinction difference of the cytochrome between 425 and 410 nm by the method of Oshino and Sato [19] with a slight modification [7, 15].

Cyclic AMP measurement: Cyclic AMP content was measured by the method of Steiner et al. [20, 21] with a cyclic AMP radioimmunoassay kit. At the indicated times cells were harvested by centrifuging the cell culture

containing over 2 x 10^7 cells/medium. Washed cells were resuspended in 5 ml of 6 % trichloroacetic acid cooled at 0-2°C. The suspension was then sonicated for 2 min and left in the ice bath for 1 h. The samples were then centrifuged at 3,000 rpm for 10 min at below 4°C, and the supernatant was washed free of trichloroacetic acid with five washes of distilled water-saturated diethyl ether and succinylated with a succinic anhydride-dioxane/triethylamine mixture (9:1, v/v). The assay mixture contained 100 µl of succinalated sample, 50 mM imidazole buffer (pH 6.5), 100 µl of anti-cyclic AMP antibody from rabbit and 16 pCi of a tyrosine methyl ester derivative of succinyl cyclic [^{125}I]AMP, and left in the cold for 18 h. The radioactivity was determined with a Packard Model 5650 Autogamma counter.

RESULTS AND DISCUSSION: Fatty acyl-CoA desaturase is associated with the multicomponent electron transport chain in microsomes of a variety of organisms, and attempts were made to elucidate the regulatory mechanism for this enzyme system. Biochemical factors such as fatty acid-binding protein [22, 23] and physiological factors such as nutrient [5, 6], temperature [1-4] and hormone [8-11] have all been implicated in regulation of activity *in vivo*. We have herein investigated the hormonal effects of dexamethasone on the fatty acyl-CoA desaturase system in *Tetrahymena* microsomes.

The data depicted in Fig. 1 shows the dual effects of dexamethasone on the Δ^9- and Δ^{12}-desaturase activities; increase of the former and decrease of the

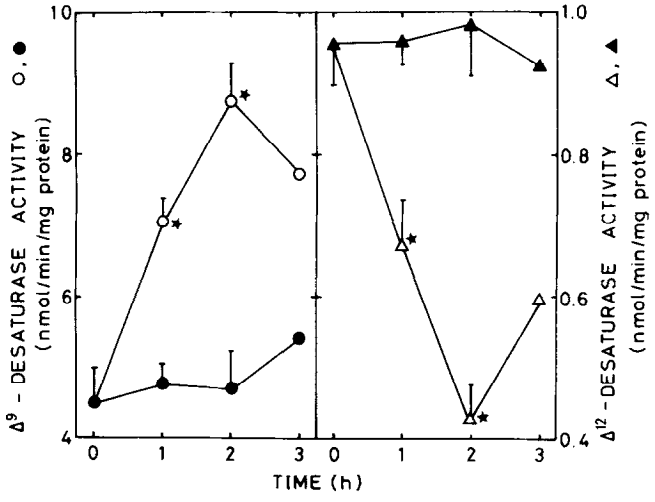

Fig. 1. Effects of dexamethasone on the fatty acyl-CoA desaturase activities in *Tetrahymena* microsomes. Details for the enzyme assays are described under "MATERIALS AND METHODS." Each point is the average (± S.D.) of 2 (or 3) independent experiments, each performed in duplicate. ○,● Δ^9-desaturase activity; △,▲ Δ^{12}-desaturase activity. ●,▲ control cells; ○, △ dexamethasone-treated cells. The asterisk indicates values differing significantly from control values by analysis of variance, $p < 0.01$.

Table I Effects of dexamethasone on the reductase activities and cytochrome b_5 content in Tetrahymena microsomes

Activity, content	After dexamethasone addition			
	0 h	1 h	2 h	3 h
	(n=3)	(n=3)	(n=3)	(n=2)
		(nmol/min/mg protein)		
NADH-ferricyanide reductase	934±82	869±64	922±68	935
NADH-cytochrome c reductase	218±16	200±11	207± 8	216
NADPH-cytochrome c reductase	15.3±2.8	15.1±0.8	15.2±2.2	16.6
		(pmol/mg protein)		
Cytochrome b_5 content	136±10	130±21	130±16	137

Details for the enzyme assays and the determination of cytochrome b_5 content are described under "MATERIALS AND METHODS." Each value is the average (± S.D.) of experiments done in duplicate. n, the number of experiments performed.

latter. This is consistent with a great incorporation *in vivo* of labeled precursors (acetate, palmitate) to oleic acid and with a significant decrease in the formation of linoleic acid (data not shown). However, dexamethasone exerted no effect on the fatty acid composition in Tetrahymena membrane lipids for at least 3 hours (data not shown).

Table I exhibits the effects of dexamethasone on the activities of intermediate electron transport components and the content of cytochrome b_5. In dexamethasone treatment of Tetrahymena, unlike temperature acclimation [1-4] and anaerobiosis [7], there was no significant differences in the content of cytochrome b_5 as well as the activities of NAD(P)H-cytochrome c and NADH-ferricyanide reductases. Nevertheless, by the first 2 hr after dexamethasone administration, the Δ^9-terminal component activity reached a peak level 2-fold higher than the control value, but the Δ^{12}-terminal component activity showed a corresponding decrease (Fig. 2). These results provide evidence that a remarkable parallelism exists in the changes of Δ^9- and Δ^{12}-desaturase and their terminal component activities, leading to suggestion that both fatty acyl-CoA desaturase activities are modulated by changes in the terminal component activity.

The profiles of the time-dependent production of cyclic AMP in control and dexamethasone-exposed cells are depicted in Fig. 3. Dexamethasone induced 3.5-fold intracellular cyclic AMP content in Tetrahymena. Generally,

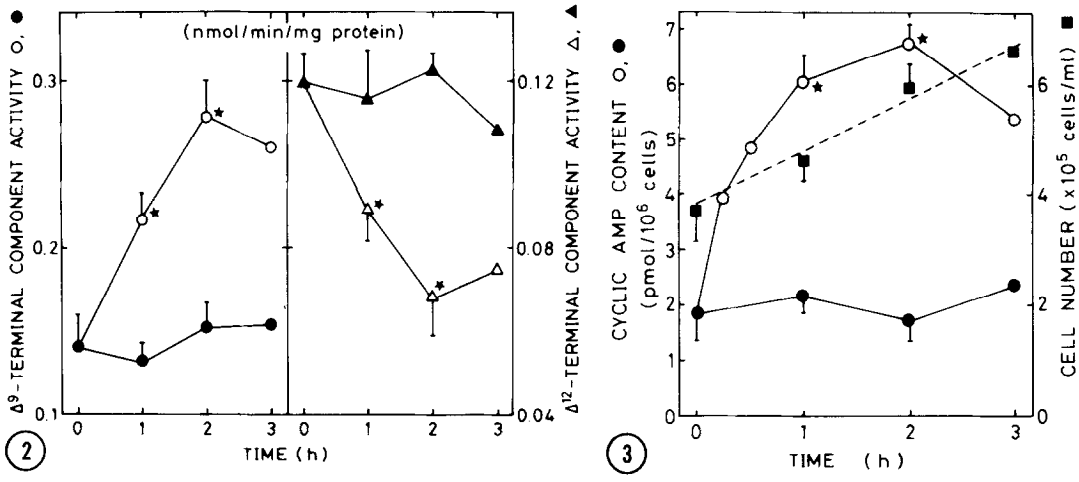

Fig. 2. Effects of dexamethasone on the activities of Δ^9- and Δ^{12}-terminal components in Tetrahymena microsomes. The activities were determined by measuring spectrophotometrically the rates of NADH-reduced cytochrome b_5 reoxidation with stearoyl-CoA and oleoyl-CoA as substrate, respectively. More details for the enzyme assay are described under "MATERIALS AND METHODS." ○,● Δ^9-terminal component activity; △,▲ Δ^{12}-terminal component activity. ●,▲ control cells; ○,△ dexamethasone-treated cells. Each point is the average (± S.D.) of 2 (or 3) independent experiments done in duplicate. The asterisk indicates values differing significantly from control values, $p < 0.01$.

Fig. 3. Effects of dexamethasone on cyclic AMP production by Tetrahymena. Detials for cyclic AMP measurement are described under "MATERIALS AND METHODS." At the indicated incubation times, cells were harvested by cenrifugation. ●—●, control cells; ○—○, dexamethasone-treated cells. Each point is the average (± S.D.) of 2 (or 3) independent experiments done in duplicate. ——, cyclic AMP content; – – –, cell number. The asterisk indicates values differing significantly from control values, $p < 0.01$.

glucocorticoids have been shown to evoke an increase of intracellular cyclic AMP accumulation by their inhibition of cyclic AMP-phosphodiesterase activity or by a secondary effects on cell nucleus [24, 25]. Based on the above observations, it may be concluded that dexamethasone would affect the fatty acyl-CoA desaturase activity in Tetrahymena microsomes by increasing intracellular cyclic AMP content. In the earlier report studying the Δ^9-, Δ^6- and Δ^5-desaturation activities in rat liver microsomes under glucocorticoid treatment, de Gómez Dumm et al. [11] have propsed a hypothesis that a glucose metabolite, which accumulates due to inhibition by glucocorticoids of the enzymes involving glucose degradation, may alter the activity of microsomal fatty acid desaturation. However, at the present moment we have no plausible interpretation for the dual effects of dexamethasone on Δ^9- and Δ^{12}-desaturase activities; increase of the former

and decrease of the latter. We have recently obtained the finding that glucose and β-adrenergic agents, such as epinephrine and isoproterenol, induced changes in Δ^9- and Δ^{12}-desaturase activities and also in intracellular cyclic AMP content [Umeki and Nozawa, manuscript in preparation]. Then we postulated a hypothetical concept that the fatty acyl-CoA desaturase system in Tetrahymena microsomes may be controlled by a cyclic AMP-dependent phosphorylation-dephosphorylation mechanism, as observed with acetyl-CoA carboxylase [26] and 3-hydroxy-3-methylglutaryl-CoA reductase [27].

In this study, we have aimed at understanding the hormonal control of oxidative desaturation system in microsomes. According to the results reported in the literature, we can assume that dexamethasone appears to participate in a cyclic AMP-dependent phosphorylation-dephosphorylation mechanism of the desaturase enzymes, especially terminal component. However, further studies are necessary to clarify the precise mechanism of action of glucocorticoids on fatty acid desaturating enzymes.

ACKNOWLEDGEMENT: This work was supported in part by grant from the Ministry of Education, Science and Culture, Japan.

REFERENCES:

1. Martin, C.E., Hiramitsu, K., Kitajima, Y., Nozawa, Y., Skriver, L. and Thompson, G.A. (1976) Biochemistry 15, 5218-5227.
2. Nozawa, Y. and Kasai, R. (1978) Biochim. Biophys. Acta 529, 54-66.
3. Umeki, S., Fukushima, H., Watanabe, T. and Nozawa, Y. (1982) Biochem. Int. 4, 101-107.
4. Umeki, S., Maruyama, H. and Nozawa, Y. (1983) Biochim. Biophys. Acta 752, 30-37.
5. Oshino, N. and Sato, R. (1972) Arch. Biochem. Biophys. 149, 369-377.
6. Nozawa, Y., Kasai, R. and Sekiya, T. (1980) Biochim. Biophys. Acta 603, 347-365.
7. Umeki, S. and Nozawa, Y. (1983) Biochim. Biophys. Acta, in press.
8. Joshi, V.C. and Aranda, L.P. (1979) J. Biol. Chem. 254, 11779-11782.
9. Kasturi, R. and Joshi, V.C. (1982) J. Biol. Chem. 257, 12224-12230.
10. Faas, F.H. and Carter, W.J. (1981) Biochem. J. 193, 845-852.
11. De Gómez Dumm, I.N.T., de Alaniz, M.J.T. and Brenner, R.R. (1979) J. Lipid Res. 20, 834-839.
12. Nozawa, Y. (1975) Methods in Cell Biology, Vol. X, pp. 105-133, Academic Press, New York.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
14. Oshino, N., Imai, Y. and Sato, R. (1966) Biochim. Biophys. Acta 128, 13-28.
15. Umeki, S., Fukushima, H. and Nozawa, Y. (1983) J. Therm. Biol. 8, 353-360.

16. Yonetani, T. (1965) J. Biol. Chem. 240, 4509-4514.
17. Schellenberg, K.A. and Hellerman, L. (1958) J. Biol. Chem. 231, 547-556.
18. Fukushima, H., Umeki, S., Watanabe, T. and Nozawa, Y. (1982) Biochem. Biophys. Res. Commun. 105, 502-508.
19. Oshino, N. and Sato, R. (1971) J. Biochem. 69, 169-180.
20. Steiner, A.L., Kipnis, D.M., Utiger, R. and Parker, C.W. (1969) Proc. Natl Acad. Sci. U.S.A. 64, 367-373.
21. Steiner, A.L., Parker, C.W. and Kipnis, D.M. (1972) J. Biol. Chem. 247, 1106-1113.
22. Catala, A.p Nervi, A.M. and Brenner, R.R. (1975) J. Biol. Chem. 250, 7481-7484.
23. Jeffcoat, R., Brawn, P.R. and James, A.T. (1976) Biochim. Biophys. Acta 431, 33-44.
24. Manganiello, V. and Vaughan, M. (1972) J. Clin. Invest. 51, 2763-2767.
25. Thompson, W.J. and Williams, R.H. (1974) Arch. Biochem. Biophys. 165, 468-477.
26. Inoue, H. and Lowenstein, J.M. (1972) J. Biol. Chem. 247, 4825-4832.
27. Beg, Z.H. and Brewer, H.B., Jr. (1982) Fed. Proc. 41, 2634-2638.