ACTH depresses Δ^6 and Δ^5 desaturation activity in rat adrenal gland and liver

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Abstract The in vivo and in vitro effect of ACTH on the biosynthesis of polyunsaturated fatty acid of rat adrenal gland and liver was studied. The administration of ACTH to intact rats produced a significant decrease in the conversion of [1-^{14}C]linoleic acid to γ-linolenic acid, [1-^{14}C]α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid, and [1-^{14}C]eicosa-8,11,14-trienoic acid to arachidonic acid in liver and adrenal microsomes. Isolated adrenocortical cells and hepatocytes obtained from animals treated with ACTH showed a decrease in the incorporation and desaturation of exogenous [1-^{14}C]eicosa-8,11,14-trienoic acid. The addition of ACTH to the incubation medium of adrenocortical cells and hepatocytes isolated from untreated rats also caused a decrease in Δ^5 desaturation activity. The effect of ACTH on adrenal and liver desaturases could be produced as a consequence of the release of glucocorticoids, already measured in the experiments. However, the in vitro experiments carried out with hepatocytes isolated from untreated rats, where corticosterone was absent, indicated that ACTH can depress Δ^5 desaturation per se.—**Mandon, E. C., I. N. T. de Gómez Dumm, M. J. T. de Alaníz, C. A. Marra,** and **R. R. Brenner.** ACTH depresses Δ^6 and Δ^5 desaturation activity in rat adrenal gland and liver. *J. Lipid Res.* 1987. **28:** 1377–1383.

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It is known from preliminary studies that epinephrine regulates the biosynthesis of polyunsaturated fatty acids in liver and adrenal gland microsomes of the rat (1–3). A single dose of this hormone administered to normal animals produces a significant decrease in Δ^6 and Δ^5 desaturase activity in both tissues. In the liver this action was attributed to an increase of the intracellular levels of cyclic AMP (1) and operated by a β-adrenergic mechanism (4). Besides, preliminary findings in work with either isolated hepatocytes or adrenocortical cells suggest that epinephrine does not produce a direct effect on arachidonic acid biosynthesis (2, 5). Therefore, it is probable that epinephrine acts in an indirect way and that the possible mechanism of action of this hormone could be through ACTH release.

It is well established that many stimuli that activate the sympathetic-adrenomedullary system also activate the pituitary-adrenocortical axis. Thus, the systemic administration of epinephrine stimulates adrenocortical activity in animals (6–9) and in humans (10) through the release of ACTH from the pituitary gland. This effect is mediated by a β-adrenergic receptor (9), and has also been shown directly in the rat anterior pituitary cells in culture (11, 12).

Taking into account the aforementioned considerations, it is possible that the effect of epinephrine on the biosynthesis of polyunsaturated fatty acids, at least in the adrenal gland, is mediated through the release and subsequent action of ACTH. Therefore, in the present study we have investigated the in vivo and in vitro effect of ACTH on the oxidative desaturation of fatty acids in the liver and adrenal gland of intact rats.

MATERIALS AND METHODS

Chemicals

[1-^{14}C]Palmitic acid (56.0 mCi/mmol), [1-^{14}C]linoleic acid (52.6 mCi/mmol), [1-^{14}C]α-linolenic acid (51.0 mCi/mmol, and [1-^{14}C]eicosa-8,11,14-trienoic acid (54.9 mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA. All the acids were 98–99% radiochemically pure. NADH, ATP, CoA, bovine albumin (essentially fatty acid-free), collagenase type IV, and deoxyribonuclease I were obtained from Sigma Chemical Corp., St. Louis, MO. All other chemicals used were of analytical grade. Dexamethasone phosphate (Dx) was obtained from Merck Sharp and Dohme and adrenocorticotropin (ACTH) was from Elea Laboratories, Argentina.

Abbreviations: Dx, dexamethasone phosphate.

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Treatment of the animals

Female Wistar rats (150–200 g body weight) maintained on a commercial diet (Cargill type C) and water ad libitum were used. All the rats were fasted for 24 hr, refed with the commercial diet for 2 hr, and killed 12 hr after the refeeding period.

The rats were divided into four experimental groups (control, treated with ACTH, treated with Dx, and treated with ACTH plus Dx). ACTH (3 IU per rat) was administered intraperitoneally 30 min prior to decapitation. Dx was given ad libitum overnight by adding 10 μg/ml in the drinking water at the end of the refeeding period. Control and Dx groups received saline solution in place of ACTH.

Isolation of microsomes and enzyme assays

Rats were killed by decapitation and the blood was allowed to drain and was collected for corticosterone determination (13). Livers from three rats per group, processed individually, and adrenal glands pooled from 24 rats per group were used to obtain microsomes. Livers and adrenal glands (free of fat and decapsulated) were rapidly excised, weighed, and placed in ice-cold homogenizing solution (1:3, w/v) containing 0.25 M sucrose, 62 mM phosphate buffer, pH 7.0, 0.15 M KCl, 5 mM $MgCl_2$, and 100 μM EDTA. The microsomal fractions were separated by differential centrifugation at 105,000 *g* as described previously (14). Protein content was measured by the method of Lowry et al. (15).

The desaturation of the fatty acids of liver and adrenal microsomes was measured by estimation of the percentage conversion of [1-^{14}C]linoleic acid to γ-linolenic acid, [1-^{14}C]linolenic acid to octadeca-6,9,12,15-tetraenoic acid and [1-^{14}C]eicosa-8,11,14-trienoic acid to arachidonic acid. Three nmol of the labeled acid and 97 nmol of unlabeled acid were incubated with 5 mg of liver microsomal protein. Adrenal microsomal Δ^6 desaturation was measured by incubating 1.5 mg of protein and 20 nmol (13.4 μM) of labeled linoleic or α-linolenic acids. The Δ^5 desaturation activity was measured with 1 mg of adrenal microsomal protein and 15 nmol of labeled eicosa-8,11,14-trienoic acid by the procedure described in a previous work. Under these experimental conditions the desaturation activity was linearly related to protein concentration and the enzymes were completely saturated with the corresponding substrates (3). The incubation medium contained 4 μmol of ATP, 0.1 μmol of CoA, 1.25 μmol of NADH, 5 μmol of $MgCl_2$, 2.42 μmol of n-acetyl cysteine, 62.5 μmol of NaF, 0.5 μmol of nicotinamide, and 62.5 μmol of phosphate buffer (pH 7) in a total volume of 1.5 ml of 0.15 M KCl, 0.25 M sucrose solution, per tube.

The tubes containing substrate and cofactors were placed in a metabolic shaker for 60 sec at 37°C; then the reaction was started by addition of the microsomal suspension. The incubation was carried out for 10 min and the reaction was stopped by the addition of 2 ml of 10% KOH in ethanol. After 45 min of saponification at 85°C under nitrogen, the acidified solution was extracted with petroleum ether (bp 30–40°C). The preparation of the fatty acid methyl esters and the procedure followed to analyze the distribution of the radioactivity between substrate and product was determined as detailed in a previous study (5) except for the packing of the radiochromatography column (10% SP-2330 on Chromosorb WAW-DMCS (100–200 mesh) Supelco Inc., Bellefonte, PA).

Preparation of adrenocortical cells and isolation of hepatocytes

To obtain adrenocortical cells, lots of three animals each were separated into the groups described above (control, treated with ACTH, treated with Dx, and treated with ACTH plus Dx). The adrenal glands of each group of rats were removed immediately after decapitation and were pooled and kept in cold Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, which contained 0.2% glucose and 0.5% albumin. The adrenals were decapsulated and the isolation of the cell population from the zona fasciculata-reticularis was obtained by mechanical agitation in siliconized glassware with collagenase and deoxyribonuclease, according to the procedure of Ray and Strott (16). The pellets of the adrenocortical cells were resuspended in KRB containing 0.2% glucose. The entire procedure of adrenocortical cell isolation took about 90 min.

Liver parenchymal cells were obtained from individual animals that were treated as indicated above. The isolation of hepatocytes was performed by perfusing the liver with collagenase according to Seglen's method (17) except that the operational temperature was maintained at 37°C throughout. To minimize glycogenolysis, 2 g of glucose per liter was added to all media employed during the perfusion and isolation (18). The pellets of the isolated hepatocytes were resuspended in IMEM-Zo medium (19) in which $CaCl_2$ concentration was lowered to 1.4 mM and linoleic acid was omitted.

Both kinds of cells were counted in a hemocytometer. Viability of dissociated cells was assessed by exclusion of trypan blue (20) and was routinely 85 to 90%.

Assay procedure

Aliquots of hepatocytes (3×10^6 cells) and adrenocortical cells (80,000 cells) were distributed in siliconized incubation flasks. The same medium utilized to resuspend the cells was added to obtain a final volume of 5 ml for the hepatocytes and 1.5 ml for the adrenocortical cells. The sodium salt of [1-^{14}C]eicosa-8,11,14-trienoic acid (80 μM concentration for the hepatocytes, and 6.7 μM concentration for the adrenocortical cells) was added bound to defatted albumin according to Spector, Steinberg, and Tanaka (21).

The incubations were performed in a metabolic shaker (80 oscillations/min) under 95% O_2 and 5% CO_2 at 37°C for 3 hr.

At the end of the incubation, the cell suspensions were transferred to cold tubes and rapidly centrifuged. Aliquots of the incubation medium of adrenocortical cells were separated to measured corticosterone levels (13). The cell pellets of both kinds of cells were washed twice with cold 0.85% NaCl solution and then resuspended in the same solution. An aliquot of the suspensions was used to measure the amount of cellular protein (15). The remainder was centrifuged at 2,000 *g* for 5 min. The saline solution was decanted and the cells were saponified and esterified as indicated above. The radioactivity of the recovered methyl esters was determined in a Beckman liquid scintillation counter (Model L5-3133P) with 96.7% efficiency for ^{14}C. The estimation of distribution of radioactivity between the fatty acids and the calculation of the percentage conversion were performed as stated above, except that a gas-liquid radiochromatograph equipped with a Panax proportional counter and a Suroscribe 2 S inscriptor were used.

In vitro assay of ACTH effect on isolated cells

Additional control groups of rats were used to isolate either adrenocortical cells or hepatocytes. The adrenal glands of six rats were pooled to obtain the isolated cells as indicated above. In other groups of four rats, livers were perfused with collagenase to separate hepatocytes as described above. Isolated adrenocortical cells or hepatocytes were incubated with [1-^{14}C]eicosa-8,11,14-trienoic acid in the presence or absence of ACTH (250 μU per flask), for 3 hr under the conditions already established.

Student's *t* test was used for the statistical treatment of the data.

RESULTS

Effect of ACTH administration in vivo on Δ^6 and Δ^5 desaturating activity of liver and adrenal microsomes

The results of this experiment are shown in **Fig. 1 (a, b, and c)**. Injection of ACTH in rats produced a significant decrease in the conversion of [1-^{14}C]linoleic acid to γ-linolenic acid (a), [1-^{14}C]α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid (b), and [1-^{14}C]eicosa-8,11,14-trienoic acid to arachidonic acid (c), in liver and adrenal gland microsomes. The depression was greater in the adrenal gland than in the liver. In the adrenal gland the desaturases showed about a 50% decrease. Dx in the drinking water also produced a strong inhibition on the desaturating activities studied. In this case the depression of Δ^6 desaturase was more evident in the liver than in the adrenal gland. When the rats injected with ACTH were pretreated with Dx, a greater decrease in the Δ^6 and Δ^5 desaturating activity was observed in both tissues.

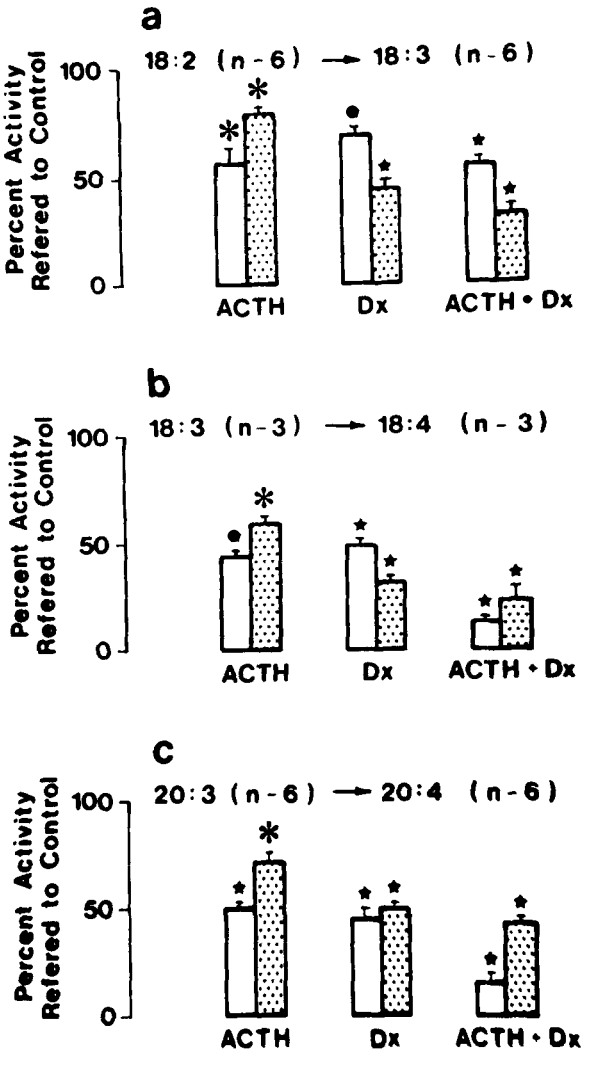

Fig. 1. Effect of ACTH and Dx, administered to intact rats, on the conversion of linoleic acid to γ-linolenic acid (a), α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid (b) and eicosa-8,11,14-trienoic acid to arachidonic acid (c) in adrenal gland □ and liver ⊠ microsomes. One hundred percent represents activities (nmol of substrate converted/mg of protein per min) in controls. The actual control activities are a: adrenal 0.204 ± 0.003, liver 0.257 ± 0.012; b: adrenal 0.502 ± 0.045, liver 0.466 ± 0.031; c: adrenal 0.860 ± 0.024, liver 0.798 ± 0.076. Results are the means of three experiments and vertical lines represent ± SEM. Means significantly different from the respective controls (* $P < 0.01$; ● $P < 0.001$).

Incorporation and desaturation of [1-^{14}C]eicosa-8, 11,14-trienoic acid in isolated adrenocortical cells and hepatocytes obtained from rats treated with ACTH

These results are shown in **Fig. 2 (a and b)**. When eicosatrienoic acid was added to the incubation medium, the isolated adrenocortical cells obtained from rats treated

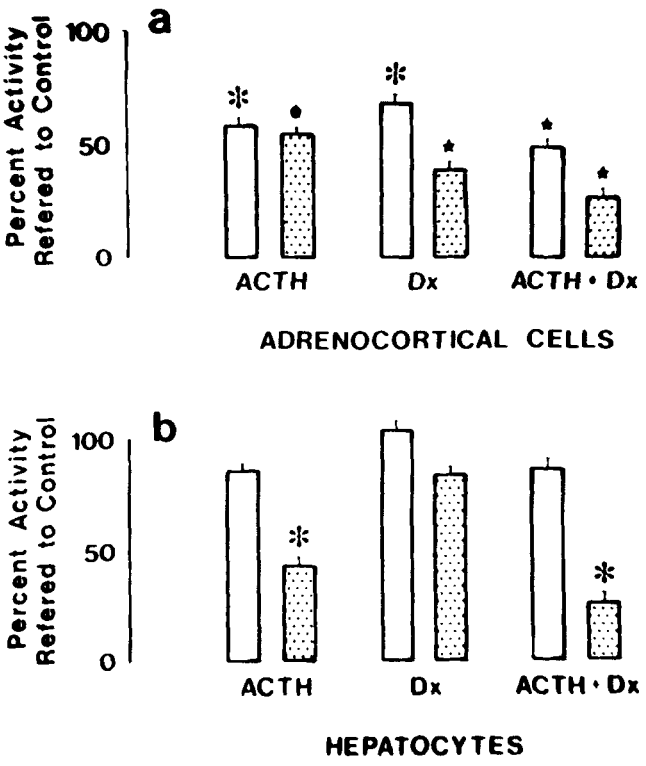

Fig. 2. Effect of ACTH and Dx, administered to intact rats, on the change of the incorporation □ and desaturation ⊡ of eicosa-8,11,14-trienoic acid in isolated adrenocortical cells and hepatocytes after a 3-hr incubation. One hundred percent corresponds to control values: 2.293 ± 0.017 nmol/mg of protein incorporated and 0.877 ± 0.022 nmol/mg of protein desaturated by adrenocortical cells (a) and 63.5 ± 0.5 nmol/mg protein incorporated and 3.5 ± 0.2 nmol/mg protein desaturated by the isolated hepatocytes (b). The incubation conditions were as described in the assay procedure. Values are the means of four incubation flasks ± SEM. Means significantly different from the respective controls (*$P < 0.01$; ★$P < 0.001$).

with ACTH showed a decrease of both the incorporation and desaturation of the labeled acid (a). The administration of Dx in the drinking water also produced a decrease in both parameters. When the rats injected with ACTH were pretreated with Dx, an additive effect was shown and a greater decrease in the incorporation and desaturation of 20:3 (n-6) was observed. In this case, the conversion of 20:3 (n-6) to arachidonic acid was more pronouncedly decreased than the incorporation of the acid.

Fig. 2 b shows the effect of ACTH administration to the rats on the incorporation and desaturation of eicosatrienoic acid in isolated hepatocytes. The incorporation of the labeled acid was not modified significantly in the hepatocytes obtained from the different groups of rats. However, the treatment of the animals with ACTH produced a very significant decrease in the Δ^5 desaturation of the acid. This hormonal effect was potentiated when the rats were previously treated with Dx, but Dx alone only produced a 16% decrease in the desaturation of the acid.

Effect of the addition of ACTH to the incubation medium of isolated adrenocortical cells and hepatocytes obtained from untreated rats

To test whether the inhibitory effect of ACTH on the Δ^5 desaturase is produced in cells isolated from untreated rats, ACTH was added to the incubation medium. **Fig. 3 (a and b)** shows the incorporation and desaturation of [1-^{14}C]eicosa-8,11,14-trienoic acid in the isolated cells 3 hr after the addition of ACTH to the incubation medium. In the adrenocortical cells both parameters decreased significantly. However, the inhibition in the production of arachidonic acid was more significant. The addition of ACTH to the incubation medium of hepatocytes did not modify the incorporation of eicosatrienoic acid, while a significant decrease in the biosynthesis of arachidonic acid was observed as compared with cells incubated in the absence of the hormone.

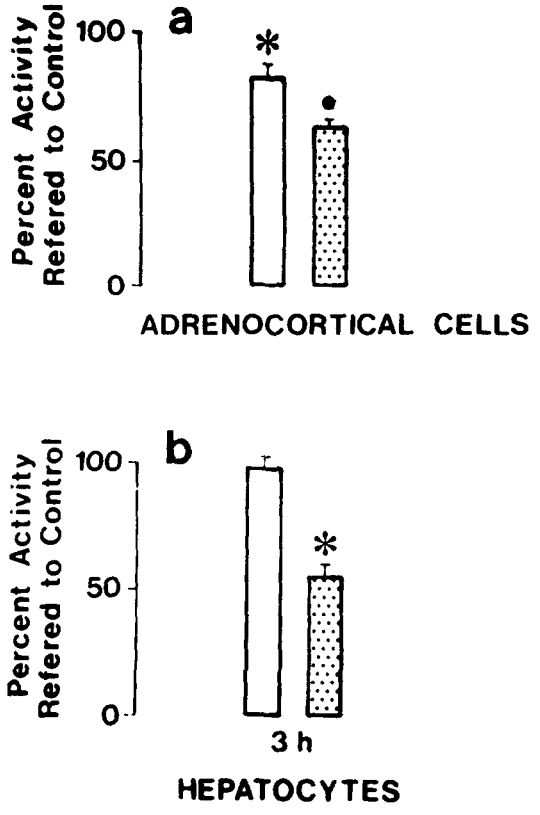

Fig. 3. Effect of the addition of ACTH to the incubation medium on the change of the incorporation □ and desaturation ⊡ of eicosa-8,11,14-trienoic acid in isolated adrenocortical cells and hepatocytes obtained from untreated rats. One hundred percent corresponds to 2.154 ± 0.035 nmol/mg of protein incorporated and 0.789 ± 0.019 nmol/mg of protein desaturated by adrenocortical cells (a) and 69.0 ± 0.7 nmol/mg of protein incorporated and 9.9 ± 0.4 nmol/mg of protein desaturated by the isolated hepatocytes (b). The incubation conditions were as described in the assay procedure. Values are the means of four incubation flasks ± SEM. Means significantly different from the respective controls (*$P < 0.01$; ★$P < 0.01$).

Corticosterone levels in rat plasma and in the incubation medium of isolated adrenocortical cells

Since adrenal cells produce corticoids and glucocorticoids inhibit Δ^5 desaturase (22) it was important to measure the secretion of corticosterone during the experiments. **Table 1** shows that the administration of ACTH to normal rats produced an increase in the plasma corticosterone levels. In addition, the cells obtained from the rats treated with ACTH also maintained the capacity to produce corticosterone after the isolation procedure, since the level of the steroid after 3 hr of incubation was twice that of the control medium. When the rats were simultaneously treated with Dx, plasma corticosterone levels were not different from those obtained in the rats injected with ACTH. However, the production of the steroid by the isolated cells was higher.

No detectable changes of corticosterone levels were found in plasma or in the incubation medium when the animals were maintained under Dx treatment, as compared with the controls.

Table 2 shows the corticosterone levels in the incubation medium of adrenocortical cells obtained from untreated rats and incubated for 3 hr. The addition of ACTH to the medium produced a significant increase in the corticosterone levels compared to the control.

DISCUSSION

From the results obtained in these experiments (Fig. 1), it is evident that the pituitary hormone ACTH produces a decrease in microsomal Δ^6 and Δ^5 desaturation activity in liver and adrenal gland of rats, modulating the biosynthesis of polyunsaturated fatty acids in these organs. Considering that the desaturases are regulated by hormones, that polyunsaturated fatty acids of 20 and 22 carbons are present in significant concentrations in the adrenal glands, and that these glands are the major target organs of ACTH, it is not unexpected that ACTH modifies polyunsaturated fatty acid biosynthesis in these cells.

TABLE 1. Corticosterone levels of rat plasma and incubation medium of isolated adrenocortical cells from treated animals

Treatment	Plasma	Incubation Medium of Isolated Cells
	μg/dl	*μg/8 × 10⁴ cells*
Control	10.7 ± 4.0[a]	0.080 ± 0.009
ACTH	42.0 ± 7.4[a]	0.167 ± 0.019[a]
Dx	8.2 ± 4.6	0.084 ± 0.010
Dx + ACTH	47.4 ± 9.3[a]	0.282 ± 0.018[b]

Each value represents the mean ± SEM.
[a]Means significantly different from controls, $P < 0.01$.
[b]Means significantly different from controls, $P < 0.001$.

TABLE 2. Corticosterone levels in the medium of adrenocortical cells incubated with ACTH

Incubation Conditions	Corticosterone Concentration
	μg/8 × 10⁴ cells
Control	0.083 ± 0.023
ACTH	0.613 ± 0.031[a]

Each value represents the mean ± SEM.
[a]Means significantly different from the controls, $P < 0.01$.

However, this effect was also shown in liver in which the existence of receptors for ACTH has not been reported.

The effect of ACTH, administered to the whole animal, was observed not only in the microsomes but also in the isolated adrenocortical and liver cells (Fig. 2). In adrenocortical cells a decrease in the incorporation and desaturation of eicosatrienoic acid was observed, indicating that the hormone triggers a mechanism that results in the depression of the desaturases within 30 min of ACTH administration. The inhibition of the desaturases persists in the isolated cells for at least approximately 5 hr. The depression of the labeled acid incorporation in the adrenals indicates that the amount of the precursor inside the cells could be the limiting step in the desaturation and then the inhibition of the desaturation would be a consequence of the depression in the incorporation of the acid. However, this is not the case since the depression of the biosynthesis of arachidonic acid evoked by ACTH is higher than that produced on the incorporation of eicosatrienoic acid into the cells (Fig. 2). The dissociation between the incorporation and the desaturation of eicosatrienoic acid was more evident in the experiments with hepatocytes. Moreover, an inhibition in liver and adrenal microsomes of Δ^5 desaturase activity was also observed in liver and adrenal microsomes of rats treated with ACTH (Fig. 1).

All these results suggest that the effect of ACTH on the adrenal and liver desaturase could be produced as a consequence of the release of glucocorticoids. In this respect it was demonstrated that the in vivo administration of glucocorticoids strongly depresses the activity of Δ^6 and Δ^5 desaturases in liver microsomes (22). Moreover, Table 1 shows that ACTH produces a significant increase in plasma corticosterone levels and that the adrenal cells isolated from the rats treated with ACTH also released corticosterone to the medium after 3 hr of incubation. Therefore, corticosterone could be the direct effector of ACTH action. However the time needed for hormonal action is different for glucocorticoids and ACTH, being significantly more rapid for ACTH. Thus the glucocorticoidal secretion hypothesis cannot explain the fast effect of ACTH. This must apparently be ascribed to the ACTH per se, since in in vitro experiments in which the hormone was directly added to the isolated hepatocytes, an

inhibition of Δ^5 desaturase was observed (Fig. 3). Hepatocytes do not produce corticosterone and hence the effect of ACTH on the depression of Δ^5 desaturase in this tissue could be caused by the hormone. However, one must also consider an additional slow effect of ACTH through the release of corticosterone when the hormone was administered to the rats or was added to the incubation medium of adrenocortical cells.

In this work we also investigated the role of endogenous ACTH production on the parameters analyzed. It is well known that Dx treatment suppresses both circadian rhythm and stress-induced output of corticosterone (23-25). For this reason, Dx was given in the drinking water of some rats. Considering that there were no differences in corticosterone plasma levels between the untreated rats and those having the pituitary adrenocortical axis inhibited by Dx (Table 1), the role of endogenous ACTH can be considered unimportant. The administration of Dx in the drinking water produced a decrease in Δ^6 and Δ^5 desaturation activity per se both in the microsomes and in the isolated cells. This effect of Dx was already observed in liver when Dx was administered to the rats by intraperitoneal injection (22) or was added to the incubation medium of isolated hepatocytes (26). From the results reported in this study, we can infer that a low dose of Dx is able to depress the activity of Δ^6 and Δ^5 desaturases. Therefore, we consider that it is not convenient to use Dx to inhibit the secretion of endogenous ACTH in order to study the biosynthesis of polyunsaturated fatty acids.

As a general conclusion, the present experiments indicate that the pituitary gland can modulate polyunsaturated fatty acid biosynthesis.

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