Effect of Epinephrine and Dibutyryl Cyclic AMP on Δ5-Desaturation Activity of Rat Liver Microsomes

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ABSTRACT

The effect of epinephrine and dibutyryl cyclic AMP on the oxidative desaturation of [1-^{14}C]-eicosatrienoic acid to arachidonic acid of rat liver microsomes has been studied. Epinephrine, at a dose of 1 mg/kg/body weight, produced a significant decrease on Δ5-desaturation activity 3 hr after the injection. This effect was maintained up to 12 hr and reached the control values 48 hr after the hormone administration. Dibutyryl cyclic AMP treatment for 24 hr (5 mg/8 hr/100 g body weight) also produced a significant decrease of the conversion of eicosatrienoic acid to arachidonic acid in rat liver microsomes. The effect of epinephrine on Δ5-desaturation activity was postulated to be evoked through an increase of the intracellular concentration of cyclic AMP.

INTRODUCTION

The regulatory effect of several hormones on the activity of the Δ6- and Δ9-desaturases of rat liver microsomes is well established. It was previously demonstrated that diabetes depressed Δ6-desaturase activity (1-3) and that low doses of insulin enhance this reaction (1,4). In addition, hormones that increase blood glucose levels, such as glucagon, epinephrine, tyroxine and glucocorticoids depress the conversion of linoleic acid to γ-linolenic acid (5-8). On the other hand, Δ9-desaturase activity increases under insulin and tyroxine treatment (7,9).

The effect of hormones on Δ5-desaturation of fatty acids is little known, therefore, this research has been designed to investigate the effect of epinephrine and dibutyryl cyclic AMP (DB cAMP) on the activity of Δ5-desaturation of fatty acids.

MATERIALS AND METHODS

Adult female Wistar rats weighing 180-220 g and maintained on standard Purina chow were used. To study the effect of a pulse of epinephrine administration, rats were divided into groups of 4 animals each. They were fasted for 24 hr and then re-fed with Purina chow for 1 hr. Water was given ad libitum. Three hr later, the rats were injected subcutaneously with epinephrine at a dose of 1 mg/kg body weight. Animals were killed by decapitation 0.5, 1.5, 3, 12, 24 and 48 hr after the injection. Blood samples were taken to measure glucose levels using the o-toluidine method (10). The rats used as controls were treated identically, except that 0.9% saline was substituted for epinephrine.

In other experiments, the effect of dibutyryl cyclic AMP on Δ5-desaturation activity of rat liver microsomes was tested. The rats were assembled into 2 groups of 5 animals each. One group was injected intraperitoneally with dibutyryl cyclic AMP (provided by Sigma Chemical Co., St. Louis, MO) at a dose of 5 mg/8 hr /100 g body weight, and the other group, used as control, was injected with 0.9% saline solution. In this experiment, the animals were fasted for 24 hr, re-fed for 2 hr with Purina chow, and then killed 12 hr after the end of the feeding period that corresponded to 24 hr after the first injection.

Microsomes were separated by differential centrifugation at 100,000 × g as described previously (8). The desaturation of eicosatrienoic acid to arachidonic acid by liver microsomes was measured by estimation of the percentage conversion of [1-^{14}C] eicosa-8,11,14-trienoic acid (54.7 mC/mmol, 98% radiochemical pure; New England Nuclear Corp., Boston, MA) to arachidonic acid. Three nmol of the labeled acid and 97 nmol of unlabeled acid were incubated with 5 mg of microsomal protein in a shaker at 35 C for 20 min. The composition of the incubation medium and the assay procedure to measure the desaturation of eicosatrienoic acid was described elsewhere (8).

RESULTS AND DISCUSSION

The effect of the administration of a pulse of epinephrine on Δ5-desaturation activity of

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eicosatrienoic acid is shown in Figure 1A. Epinephrine markedly depressed this enzymatic activity, since 3 hr after the injection the effect was highly significant. However, the depression reached the lowest value at 12 hr. Δ5-Desaturation activity was then slowly recovered, and 48 hr after the injection it was similar to the control. Blood glucose also showed a fast response to the hormone. However, the increase of the blood glucose was found 30 min after epinephrine administration and recovered to control values after 24 hr. Therefore, the effect of epinephrine on the fatty acid desaturation was slower than the effect on glycogen breakdown.

Table I shows the effect of DB cAMP on Δ5-desaturation activity of rat liver microsomes. DB cAMP highly depressed the conversion of eicosatrienoic acid to arachidonic acid. Twenty-four hr after the treatment, the activity of the enzyme was reduced to less than one-half of the original value.

In a previous study, we demonstrated that epinephrine evoked a decrease of Δ6-desaturation activity on rat liver microsomes (6). From the results obtained in this experiment, it is evident that epinephrine also modifies the activity of Δ5-desaturase, since the conversion of eicosatrienoic acid to arachidonic acid markedly decreases after the catecholamine administration. However, the decrease of Δ5-desaturase activity was not as fast as that of Δ6-desaturase. While Δ6-desaturase decreased significantly 90 min after the injection of the hormone, Δ5-desaturase activity showed the effect after 3 hr, and the recovering of the enzymes' activities in both cases were similar (Fig. 1, A and B). The change in the concentration of cAMP evoked by epinephrine injection may be seen in Figure 1B.

The effect of epinephrine on Δ6-desaturation activity was postulated to be mediated through an enhancement of the intracellular cyclic AMP levels, since it was mimicked by pharmacological activators of β receptors and inhibited by β blockers (6,11). Moreover, DB cAMP administration to intact rats also produced a significant decrease in linoleic acid desaturation activity (5). The results obtained from this experiment with Δ5-desaturase would be produced by a similar mechanism. However, in a previous study, we did not find a significant change of the Δ5-desaturation activity after treating the rats with glucagon or DB cAMP (5). On the contrary, in the experiment described here, we could demonstrate that the conversion of eicosatrienoic acid to arachidonic acid was significantly decreased, not only by epinephrine, but also by DB cAMP

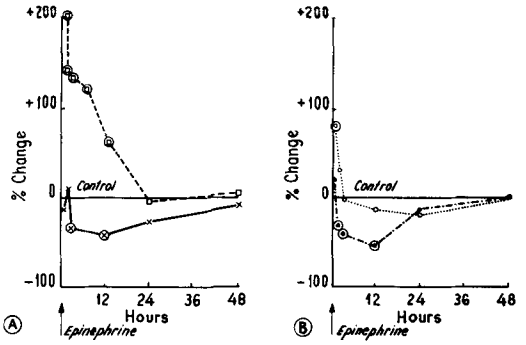

FIG. 1. (A) Effect of epinephrine administration on rat liver microsomal conversion of [1-^{14}C]eicosa-8,11,14-trienoic acid to arachidonic acid (X—X) and plasma glucose levels (□----□). Zero point corresponds to the percentage conversion (average 22.0) for eicosatrienoic acid of normal rats injected with saline solution. Each point represents the average of 4 rats. In circles, results are significantly different from the controls, $P < 0.01$. (B) Effect of epinephrine administration on rat liver microsomal conversion of [1-^{14}C]-linoleic acid to γ-linolenic acid (·—·—·) and liver cAMP levels (o·····o). Zero points corresponds to the percentage conversion (average 21.7) for linoleic acid of normal rats injected with saline solution. Each point represents the average of 4 rats. In circles, results are significantly different from the controls, $P < 0.05$ for cAMP levels and < 0.01 for linoleic acid conversion to γ-linolenic acid. Results calculated from de Gómez Dumm et al. (6).

treatment. This apparent difference could be explained by the different experimental designs. In the experiment just mentioned, the rats were re-fed with a fat-free diet while they were simultaneously treated with DB cAMP, whereas in the present experiment, they were fed on Purina chow. It has been demonstrated that rats maintained on a fat-free diet show a significant decrease of the Δ5-desaturation activity when compared to animals fed a balanced diet (12). Therefore, the fat-free refeeding diet would have masked the decrease of Δ5-desaturation activity produced by DB cAMP

TABLE I

Effect of Dibutyryl Cyclic AMP on Δ5-Desaturation Activity of Rat Liver Microsomes[a]

	20:3→20:4	
	Conversion (%)	
Control	18.0 ± 1.7[b]	$P < 0.01$
DB cAMP	7.6 ± 1.1	

[a]See Materials and Methods for details.

[b]Averages of the analyses of 5 rats ± 1 standard error of the mean.

administration (5).

The results obtained in this work show that Δ5-desaturase activity of rat liver microsomes might be controlled by endocrinological factors. The effect of epinephrine on fatty acids Δ5- and Δ6-desaturases would be similar and it would be produced by an increase of the intracellular levels of cyclic AMP.

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