Effect of epinephrine on the oxidative desaturation of fatty acids in the rat

Irma N. T. de Gómez Dumm, Maria J. T. de Alaniz, and Rodolfo R. Brenner[1]

Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de la Plata, Calle 60 y 120, La Plata, Argentina

Abstract The effect of epinephrine on the oxidative desaturation of fatty acids by liver microsomal preparations of rats has been studied. Administration of epinephrine (1 mg/kg body weight) produced a significant decrease in desaturation of [1-^{14}C]linoleic acid to γ-linolenic acid and of [1-^{14}C]α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid 12 hr after the injection. Lower doses produced a lesser effect on the Δ6-desaturation activity. Epinephrine administration modified the V_{max} of linoleic acid desaturation but not the K_m. There was also a slight increase in palmityl desaturation activity. The effect of epinephrine on Δ6-desaturation activity was postulated to be mediated through an enhancement of the intracellular cyclic AMP levels that lead to an increase of a glucose metabolite. This metabolite would inhibit Δ6-desaturation activity.

Supplementary key words palmitic acid · linoleic acid · α-linolenic acid · glucose metabolism · liver microsomes · cyclic AMP

The oxidative desaturation of fatty acids in mammals is under hormonal control. Insulin has been considered to be involved in the regulation of Δ9-, Δ6-, and Δ5-desaturations. Gellhorn and Benjamin (1) showed that the oxidative desaturation of stearic to oleic acid is depressed in the microsomes of the alloxan-diabetic rat. The same defect was found by Mercuri, Peluffo, and Brenner (2, 3) in the activity of the Δ6-desaturase. When the diabetic rats were injected with insulin the desaturating activity of the acids was recovered. The Δ5-desaturation of eicosadienoic acid was decreased by alloxan-diabetes to a lesser extent than the Δ6-desaturation (4).

Fasting causes a decrease of the Δ6- and Δ9-desaturation activities of rat liver microsomes (5, 6). Refeeding enhanced the activity of both enzymes (6, 7), but the administration of glucagon or dibutyryl cyclic AMP abolished the increase of the Δ6-desaturase activity elicited by refeeding, while no effect was found in the Δ9- or Δ5-desaturation activities (8). The effect of glucagon was considered to be produced through a direct or indirect action of cyclic AMP on the desaturating enzyme. Since the primary action of catecholamines on the liver is the stimulation of adenyl cyclase and the formation of 3′5′ cyclic AMP (9, 10), the aim of the present experiment was to study the influence of epinephrine on the oxidative desaturation of palmitic, linoleic, and α-linolenic acids.

MATERIALS AND METHODS

Chemicals

[1-^{14}C]Linoleic acid (56.2 mCi/mmole, 99% radiochemical purity) and 3′5′ cyclic [8-^{3}H]AMP (33.2 Ci/mmole) were purchased from New England Nuclear Corp., Boston, Mass. [1-^{14}C]Palmitic acid (37.7 mCi/mmole, 99% radiochemical purity) and [1-^{14}C]α-linolenic acid (41.5 mCi/mmole, 99% radiochemical purity) were purchased from the Radiochemical Centre, Amersham, England. NADH, ATP, CoA, cyclic 3′5′ AMP and other cofactors were provided by Boehringer Argentina.

Animals and treatment of animals

Adult female Wistar rats weighing 200–250 g and maintained on standard Purina chow were used.

Several experiments were performed. Experiment 1 was designed to show the effect of epinephrine administration to normal rats on the oxidative desaturation of linoleic and palmitic acids by liver microsomes. The rats were divided into groups of four animals each. All rats were fasted for 24 hr and then refed 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] All authors are members of the "Carrera del Investigador" of the "Consejo Nacional de Investigaciones Científicas y Técnicas", Argentina.

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1 mg/kg of body weight. Animals were killed 0.5, 1.5, 3, 6, 12, 24, and 48 hr after the injection. The rats used as controls were treated identically except for the substitution of 0.9% saline for epinephrine.

In Experiment 2 the effect of different doses of epinephrine on the oxidative desaturation of linoleic acid and α-linolenic acid was tested. The rats received the same treatment as the first group but were injected with 1, 0.5, 0.25 or 0.125 mg/kg of epinephrine and were killed 12 hr after the injection. The control group received saline solution in place of epinephrine.

Experiment 3 was designed to measure the approximate kinetic parameters of microsomal linoleic acid desaturation to γ-linolenic acid in the rats injected with 1 mg/kg of epinephrine and killed 12 hr after the treatment, and in the rats injected with saline solution. Other experimental conditions were similar to Experiment 1.

Isolation of microsomes

After the different treatment times the rats were killed by decapitation without anesthesia. The blood was allowed to drain and was collected for glucose determination. A 200–300 mg sample of liver was placed in 5% trichloroacetic acid to measure cyclic AMP concentration. The rest of the liver was excised and immediately placed in ice-cold homogenizing medium (4). After homogenization, samples were taken to measure protein and glycogen content. Microsomes were separated by differential centrifugation at 100,000 *g* as described previously (4).

Assay procedures

In the first experiment the desaturation of the fatty acids by liver microsomes was measured by estimation of the percentage conversion of [1-^{14}C]-linoleic acid to γ-linolenic acid and of [1-^{14}C]palmitic to palmitoleic acid. Three nmoles of the labeled acid and 97 nmoles of unlabeled acid were incubated with 5 mg of microsomal protein in a Dubnoff shaker at 35°C for 20 min in a total volume of 1.5 ml of 0.15 M KCl–0.25 M sucrose solution. The medium contained 4 μmoles of ATP, 0.1 μmole of CoA, 1.25 μmoles of NADH, 5 μmoles of $MgCl_2$, 2.25 μmoles of glutathione, 62.5 μmoles of NaF, 0.5 μmole of nicotinamide, and 62.5 μmoles of phosphate buffer (pH 7).

In Experiment 2, the desaturation of [1-^{14}C]linoleic to γ-linolenic acid and of [1-^{14}C]α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid were measured under the same experimental conditions described for Experiment 1.

In the third experiment 10, 25, 50, and 70 nmoles of [1-^{14}C]linoleic acid were incubated with 5 mg of microsomal protein for 10 min under the conditions of Experiment 1.

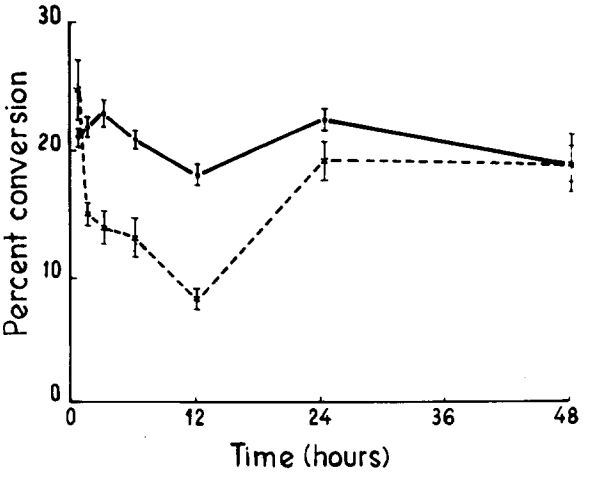

Fig. 1. Effect of epinephrine administration (1 mg/kg body wt) on the oxidative desaturation of [1-^{14}C]linoleic acid to γ-linolenic acid: epinephrine-treated (× – – – ×); controls (● —— ●). Results are the means of analysis of four animals (each analysis was performed in duplicate). Vertical lines represent 1 SEM. Results corresponding to 1.5, 3, 6, and 12 hr after epinephrine treatment are significantly different from the controls ($P < 0.01$).

In all experiments the oxidative desaturation reaction was stopped by the addition of 2 ml of 10% KOH in methanol. After 45 min of saponification at 85°C under nitrogen, the acidified solution was extracted with petroleum ether (bp 30–40°C). The fatty acids were esterified with methanolic 3 M HCl (3 hr at 68°C) and the distribution of the radioactivity between substrate and product was measured by gas–liquid radiochromatography of the methyl esters in an apparatus equipped with a Packard proportional counter. A column of 10% diethyleneglycol succinate on Chromosorb W (80–100 mesh) was used. Palmitic, linoleic, and α-linolenic acids were desaturated to palmitoleic, γ-linolenic, and octadeca-6,9,12,15-tetraenoic acids, respectively. Percentage conversion was calculated from the distribution of radioactivity between substrate and product measured directly on the radiochromatogram.

Protein content in the homogenate and in the microsomal fraction were determined by the Folin-Ciocalteu method described by Lowry et al. (11) using crystalline bovine serum albumin as standard. Blood glucose was measured by the *o*-toluidine method (12) and liver glycogen by the method of Van Handel (13). Liver preparation for cyclic AMP analysis was done according to Gilman's description (14) and cyclic AMP determination followed the method of Mato and Serrano Rios (15).

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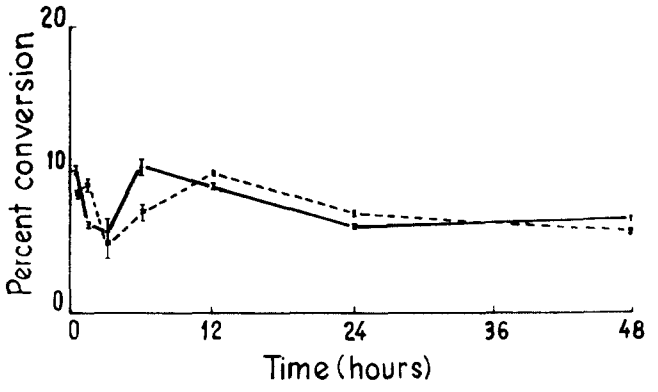

Fig. 2. Effect of epinephrine administration (1 mg/kg body wt) on the oxidative desaturation of [1-^{14}C]palmitic acid to palmitoleic acid: epinephrine treated (× – – – ×); controls (● —— ●). Results are the means of analyses performed in duplicate. Vertical lines represent 1 SEM. Results obtained after 90 min of epinephrine treatment are significantly different from the control ($P < 0.01$).

RESULTS

Effect of epinephrine administration on linoleic and palmitic acid desaturation

The effect of the administration of a pulse of epinephrine on the linoleic acid desaturation activity of rat liver microsomes is shown in **Fig. 1**. Epinephrine, at a dose of 1 mg/kg body wt, markedly depressed Δ6-desaturation of linoleic acid. After 90 min the effect was highly significant, but the depression was highest at 12 hr. After 24 hr the activity was normal and there was no significant difference compared to the control values.

Fig. 2 illustrates the effect of epinephrine on the oxidative desaturation of palmitic acid to palmitoleic acid in rat liver microsomes. In contrast to the response of linoleic acid desaturation, the palmitic acid desaturating activity increased significantly 90 min after the administration of epinephrine. However, this increase was transient and Δ9-desaturase showed no significant differences with the controls at the other times tested.

The effect of epinephrine on plasma glucose, liver glycogen, and liver cyclic AMP levels are summarized in **Table 1**. Epinephrine produced hyperglycemia and glycogenolysis. The hyperglycemia correlated with the depression of liver glycogen and was recognized at least as early as 30 min after epinephrine injection. Therefore, the effects of epinephrine on glucose metabolism are earlier than those on fatty acid desaturation (Fig. 1). After 6 hr there was hypoglycemia, which was also noted in the control group; this was due to the lack of food intake. The low glucose levels in both groups were maintained up to 48 hr. The disappearance of the epinephrine effect on glucose metabolism was earlier than that observed on Δ6-desaturation of linoleic acid (Fig. 1).

Liver cyclic AMP levels increased 30 min after the administration of the hormone. After this time the levels in both groups of animals were similar. The gradual increase in liver cyclic AMP concentration during this period may be attributed to continuous fasting (16).

Effect of different doses of epinephrine on the oxidative desaturation of linoleic and α-linolenic acids

The doses of epinephrine required to depress the desaturation of linoleic acid to γ-linolenic acid and of

TABLE 1. Effect of subcutaneous injection of epinephrine in normal rats[a]

	Plasma Glucose		Liver Glycogen		Liver cAMP	
Time	Control	Epinephrine	Control	Epinephrine	Control	Epinephrine
hr	*mg%*		*mg/mg protein*		*pmoles/mg protein*	
0.5	178[b] ± 9	306 ± 21	0.138 ± 0.010	0.093 ± 0.007	1.73 ± 0.10	3.12 ± 0.52
	$P < 0.001$		$P < 0.01$		$P < 0.05$	
1.5	146 ± 6	369 ± 20	0.135 ± 0.025	0.069 ± 0.002	2.19 ± 0.30	2.89 ± 0.45
	$P < 0.001$		$P < 0.05$			
3	119 ± 7	323 ± 15	0.135 ± 0.016	0.074 ± 0.002	2.80 ± 0.25	2.74 ± 0.10
	$P < 0.001$		$P < 0.02$			
6	130 ± 8	264 ± 19	0.140 ± 0.020	0.144 ± 0.018	2.82 ± 0.50	2.70 ± 0.10
	$P < 0.001$					
12	58 ± 8	27 ± 5	0.145 ± 0.016	0.158 ± 0.018	3.21 ± 0.30	2.82 ± 0.26
24	63 ± 2	49 ± 7	0.132 ± 0.019	0.140 ± 0.020	3.99 ± 0.67	3.25 ± 0.68
48	40 ± 5	35 ± 8	0.108 ± 0.030	0.116 ± 0.046	5.30 ± 0.64	5.40 ± 0.26

[a] 1 mg/kg body weight.
[b] Averages of the analyses of four rats ± one standard error of the mean (SEM).

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α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid are shown in **Table 2**. Epinephrine evoked an equivalent effect on both Δ6-desaturations, as is expected if we are dealing with the same enzyme. With doses of 1 mg/kg body wt, fatty acid desaturation activity decreased nearly 50% compared to the controls. Other doses also produced a decrease in the conversion of the fatty acids but the results were not statistically significant.

Effect of epinephrine on speed of [1-^{14}C]linoleic acid desaturation into γ-linolenic acid

Fig. 3 shows the approximate kinetic parameters: maximal velocity (V_{max}) and Michaelis constant (K_m) of linoleic acid desaturase in animals after 12 hr of epinephrine injection compared to controls. It was found that the administration of epinephrine (1 mg/kg body wt) produced a decrease of the approximate V_{max} from 1.54 to 0.54 × 10^{-7} M min/mg protein, but did not modify the approximate K_m (3.0 × 10^{-5} M) of the reaction.

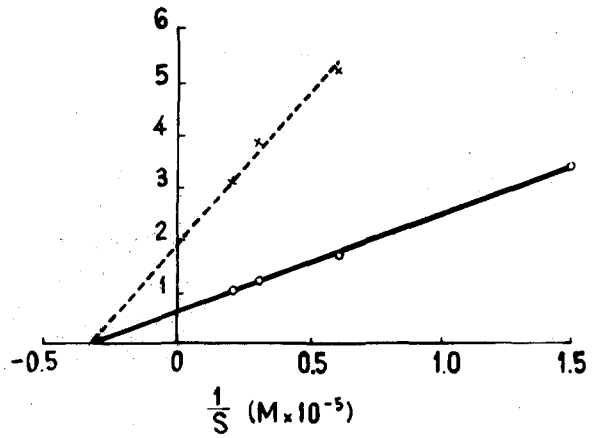

Fig. 3. Lineweaver-Burk plot of linoleic acid desaturation to γ-linolenic acid in liver microsomes of rats after 12 hr of epinephrine (× – – – ×) or saline solution (○ —— ○) injection. Approximate kinetic parameters: K_m = 3.0 × 10^{-5} M. V_{max} (control) = 1.54 × 10^{-7} M/min per mg protein; V_{max} (epinephrine) = 0.54 × 10^{-7} M/min per mg protein.

DISCUSSION

The glycogenolytic response to epinephrine represents one of the best understood actions of the catecholamines and there is no doubt that an important organ involved in this response is the liver. This effect is mediated through an elevation of the intracellular concentration of 3′5′ cyclic AMP. However, other hepatic effects of epinephrine are also produced through 3′5′ cyclic AMP such as increased ketogenesis and inhibition of the incorporation of acetate into fatty acids and cholesterol (17), stimulation of gluconeogenesis (18), depression of glycogen synthesis (19), and inhibition of the incorporation of amino acids into proteins (20). With respect to lipid metabolism there is evidence to indicate that, in the rat, epinephrine produces lipolysis acting by an increase of the intracellular concentration of cyclic AMP (21).

TABLE 2. Effect of different doses of epinephrine on the oxidative desaturation of [1-^{14}C]linoleic and [1-^{14}C]α-linolenic fatty acids[a]

Epinephrine Dose	Conversion	
	Linoleic Acid	α-Linolenic Acid
mg/kg	%	%
Control	21.8[b] ± 3.0 ($P < 0.01$)	22.8 ± 1.9 ($P < 0.01$)
1	9.8 ± 0.7	12.4 ± 0.4
0.5	15.4 ± 1.9	18.3 ± 1.2
0.25	17.6 ± 3.3	19.5 ± 0.7
0.125	17.2 ± 1.9	16.6 ± 1.9

[a] 100 nmoles of [1-^{14}C]linoleic acid or [1-^{14}C]α-linolenic acid were incubated with 5 mg of microsomal protein at 35°C for 20 min with the cofactors detailed in material and methods.

[b] Means ± one standard error of the mean of duplicate samples from four animals.

From the results obtained in this experiment it is evident that epinephrine is also involved in fatty acid Δ6-desaturation activity, since the activity of both linoleyl and α-linolenyl desaturatioin markedly decreased after the administration of the catecholamine (Table 2). It seems probable that this effect is mediated by modifications of hepatic cyclic AMP levels, since the concentration of cyclic AMP increased earlier than the decrease of the Δ6-desaturation (Table 1). Peaks of cyclic AMP have been shown (22) to be produced as early as 20 sec after epinephrine treatment. Moreover, in a recent report (8) it was described that glucagon and dibutyryl cyclic AMP inhibited the reactivation of the Δ6-desaturase that was evoked by refeeding fasted rats. This effect was considered to be either a direct consequence of an increase in the intracellular concentration of cyclic AMP or an indirect effect evoked through an increase of glucose metabolism. This last possibility was consistent with the report that showed that a glucose diet depressed the Δ6-desaturation of fatty acids (23).

Fig. 1 and Table 1 show that epinephrine evokes a decrease of Δ6-desaturase activity that begins one hr later than the rise of glucose levels and is maintained for at least 12 hr, decreasing later than the blood glucose concentration. Thus it seems likely

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that epinephrine activation of adenyl cyclase triggers a sequence of reactions evoked through an enhancement of cyclic AMP that produces an increase of some glucose metabolite. This metabolite would inhibit linoleic acid desaturation.

Fig. 3 indicates that epinephrine injection promotes only a modification of the V_{max} of linoleic acid desaturation while the K_m remains constant. Therefore, it is possible to assume that the inhibitory effect of epinephrine is evoked through a decrease in the amount of active enzyme. This effect could be evoked through an inhibition of the synthesis of the Δ6-desaturase mediated, for example, through an inhibition of insulin release (24) or through another mechanism. However, Peluffo et al. (23) have shown that approximately a 32-hr period is required for a complete fall of Δ6-desaturase activity when a rat is injected with Actinomycin D. Therefore, since the changes found in the present experiment are much faster, it is improbable that an inhibition of the enzyme synthesis could be seen in such a short period of time.

All these results may suggest that the effect of epinephrine on the Δ6-desaturase activity involves a series of reactions in which cyclic AMP is involved and is probably produced by an increase of a glucose metabolite that modifies the activity of the enzyme.

In contrast to the marked reduction of linoleyl and α-linolenyl desaturation activity observed upon epinephrine administration, palmityl desaturation activity showed only a slight modification (Fig. 2). Previously it was reported that neither glucagon nor dibutyryl cyclic AMP modified Δ9-desaturation activity (8). These results are consistent with the observations that Δ9-desaturase is different from Δ6-desaturase (25) and also that Δ9-desaturase activity is not decreased by an increase of cyclic AMP levels (8).

Although in this work there is not enough information to clarify definitively the mechanism of the action of epinephrine, it is evident that linoleic and α-linolenic acid desaturation activities are modified by this hormone. This observation is very important if we consider that the Δ6-desaturase is a regulatory enzyme that begins the synthesis of polyunsaturated fatty acids of the essential series (24) and that a reduced plasma concentration of linoleic acid is associated with human atherosclerosis and myocardial infarction (26). Moreover, we have to take into account that daily stress increases plasma levels of epinephrine.

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