THE EFFECTS OF PALMITYLCARNITINE ON HEPATIC FATTY ACID SYNTHESIS AND ON ACETYL CoA CARBOXYLASE ACTIVITY*

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During an investigation of the role of acylcarnitine formation in the regulation of the rate of long-chain fatty acid oxidation, we observed that (+)-palmitylcarnitine was a specific inhibitor for the carnitine palmityltransferase reaction (Fritz and Marquis, 1965), and that (+)-palmitylcarnitine competitively blocked the carnitine-induced increase of palmitate or palmityl CoA oxidation. It therefore appeared plausible that (+)-palmitylcarnitine might be employed to inhibit the carnitine-induced increase of fatty acid synthesis recently reported by Kipnis and Kalkhoff (1965). On the basis that palmityl CoA concentrations would probably be lowered by carnitine via operation of the carnitine palmityltransferase reaction, Kipnis and Kalkhoff (1965) suggested that decreased palmityl CoA concentrations would no longer inhibit fatty acid synthesis, thereby accounting for the observed stimulation of fatty acid synthesis by carnitine. Instead of using liver preparations from rats made acutely diabetic by injection of anti-insulin serum, we compared liver preparations from fed and starved animals. To our initial surprise, (+)-palmitylcarnitine did not inhibit fatty acid synthesis, but on the contrary greatly enhanced lipogenesis from labeled acetate. In contrast, carnitine addition stimulated lipogenesis only slightly. In this communication, we shall present our findings on the effects of (+)- and (-)-palmitylcarnitine on incorporation of acetate-1-C^{14} and malonyl-2-C^{14} CoA into fatty acids, and we shall report ancillary data indicating that the site of stimulation is probably at the level of acetyl CoA carboxylase (E. C. 6. 4. 1. 2).

METHODS

Fatty acid synthesis by 109,000 x g supernatant rat liver fractions from acetate-1-C^{14} was measured by procedures essentially identical to those reported by Abraham et al (1960). Acetate-1-C^{14} was purchased from Nuclear Chicago, and malonyl-2-C^{14} CoA was kindly provided by Dr. Roy Vagelos. Acetyl

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CoA carboxylase was obtained from pigeon livers by the methods of Waite and Wakil (1962). The preparation was carried through the second ammonium sulfate precipitation after elution of the carboxylase from calcium phosphate gel. Activity was assayed by following incorporation of C^{14}-bicarbonate into malonyl CoA (Martin and Vagelos, 1962; Waite and Wakil, 1962; Matsuhashi et al, 1964). Additional experimental details are provided in legends to tables and figures.

RESULTS AND DISCUSSION

In confirmation of the results of others (for reviews see Masoro, 1962; Fritz, 1961), starvation was associated with greatly decreased rates of fatty acid synthesis from acetate-1-C^{14}. The effects of (+)-palmitylcarnitine and (-)-palmitylcarnitine on lipogenesis by liver preparation from fed and starved rats are summarized in Fig. 1. Maximal stimulation was obtained at concentrations of (+)-palmitylcarnitine from 5×10^{-5}M to 1×10^{-4}M. In contrast, (-)-palmitylcarnitine did not appreciably enhance fatty acid synthesis at concentrations up to 5×10^{-5}M. Greatest effects were obtained in preparations from fasted rats. Carnitine (1×10^{-3}M) increased acetate incorporation only about 50% (data not shown). In one experiment on a fasted rat included in Fig. 1, 5×10^{-5}M (+)-palmitylcarnitine stimulated fatty acid synthesis from a control average of 2.4 mμmoles to a value of 52.9 mμmoles/mg liver protein/2 hrs. The average percentage increase induced by (+)-palmitylcarnitine in all experiments summarized in Fig. 1 was 475% in fed preparations and 1318% in livers from fasted rats.

It is remarkable that (+)-palmitylcarnitine increased incorporation of labeled acetate into fatty acids by livers from starved rats to an amount greater than that found in non-stimulated liver preparations from fed animals. These findings suggest that a high potential enzyme capacity for fatty acid synthesis was present, but fatty acid synthesis was indirectly inhibited during an 18 hour period of starvation by alteration of non-enzymic component(s) in the system which could be reversed by (+)-palmitylcarnitine.

In confirmation of the observations of Korchak and Masoro (1962), the incorporation of malonyl-2-C^{14} CoA into fatty acids by liver preparations from starved rats was approximately equal to that from fed animals (Fig. 2).

While (+)-palmitylcarnitine addition increased fatty acid synthesis from low concentrations of acetate-1-C^{14} by over 4-fold in preparations from fed rats, and by over 12-fold in livers from starved animals, the percentage increase in fatty acid synthesis from malonyl-2-C^{14} CoA induced by (+)-palmitylcarnitine was only 30% (Fig. 2). This latter stimulation could have

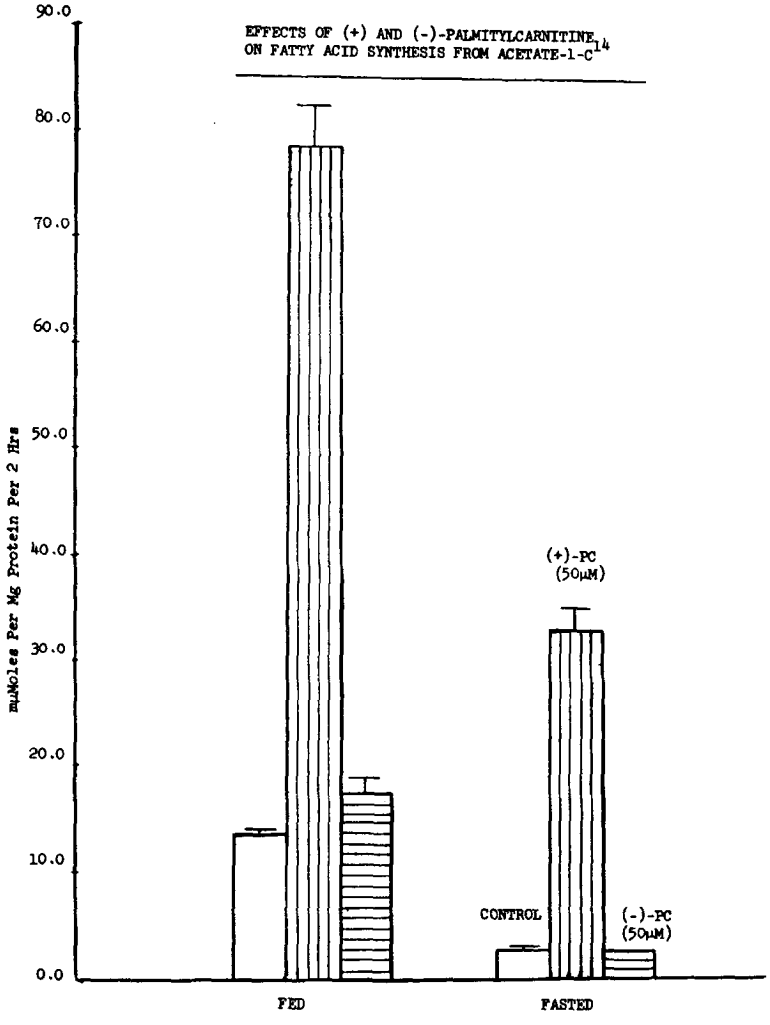

Fig. 1. Effects of (+)- and (-)-palmitylcarnitine on hepatic fatty acid synthesis from acetate-1-C^{14}. Livers were obtained from adult male Sprague-Dawley rats which had either been fed Rockland rat pellets until being sacrificed, or which had been starved for 18 hours before being killed. Livers were homogenized in loose-fitting Ten Broeck glass homogenizers (4 strokes per liver preparation of approximately 10 grams) in 3 volumes of 0.1M potassium phosphate at pH 7.5. Approximately 20 mg protein from 109,000 x g liver supernatant fractions were incubated at 37° for 2 hrs. in stoppered Ehrlermeyer flasks in a volume of 3.8 ml of the following composition: potassium citrate - 75 μmoles; ATP - 48 μmoles; CoA - 0.2 μmoles; TPN - 1 μmole; glutathione - 60 μmoles; glycylglycine buffer at pH 7.5 - 240 μmoles; $KHCO_3$ - 10 μmoles; $MnCl_2$ - 1 μmole; $MgCl_2$ - 70 μmoles; potassium phosphate at pH 7.5 - 150 μmoles; and potassium acetate-1-C^{14} - 5 μmoles containing 1.95×10^6 DPM. The gas phase was 95% O_2 and 5% CO_2. Values are the mean plus or minus the standard error of the mean for 6 to 8 separate experiments, each performed with duplicate flasks, for each group listed except the group from fasted rats treated with 50 μM (-)-palmitylcarnitine. For this group, only three separate experiments were performed.

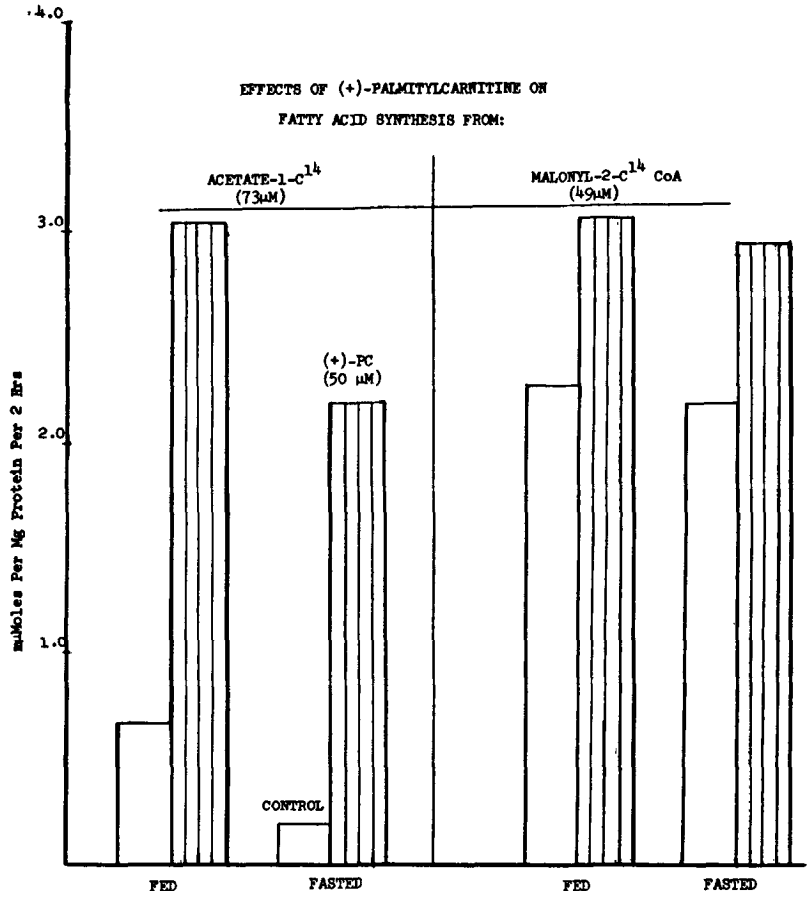

Fig. 2. Effects of (+)-palmitylcarnitine on incorporation of acetate-1-C^{14} and malonyl-2-C^{14} CoA into fatty acids by liver preparations from fed and starved rats. Concentrations and conditions were the same as those reported in Fig. 1 with the exceptions that only 10 mg protein was present in each vessel in a final volume of 2.05 ml, and substrate concentrations were lowered as indicated. Values are averages from two separate experiments, each performed in duplicate.

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resulted from a direct enhancement of fatty acid synthetase. It appears more likely, however, that a small portion of malonyl CoA was decarboxylated to acetyl CoA and then resynthesized. If the reasoning is valid, activation of acetyl CoA carboxylase could be the primary site of stimulation by (+)-palmitylcarnitine. Evidence compatible with this postulate is presented in Tables I and II.

TABLE I

Effects of Citrate and (+)-Palmitylcarnitine on Acetyl CoA Carboxylase Activity

Minutes Pre-incubation with Enzyme	Minutes After Last Component	CPM in Malonyl CoA* Control	Citrate (9.0 mM)	(+)-Palmityl-carnitine (0.05 mM)	Citrate and (+)-Palmityl-carnitine
0	15	243	457	369	817
15	15	71	540	280	880

*Pigeon liver acetyl CoA carboxylase (0.3 mg) was either added last (0 min. preincubation time) or was incubated with buffer, citrate and (+)-palmityl-carnitine for 15 min. at 30° prior to addition of remaining compounds. Final concentrations included acetyl CoA (2×10^{-4}M); ATP (4.8×10^{-3}M); $KHC^{14}O_3$ (1.0×10^{-2}M and 1.5×10^{6}CPM); mercaptoethanol (1.0×10^{-3}M); Tris HCl at pH 7.5 (1×10^{-1}M); $MnCl_2$ (1×10^{-3}M); and $MgCl_2$ (2×10^{-2}M). Stoppered tubes containing a final volume of 0.5 ml were incubated at 30° for 15 minutes after addition of the last component and the reaction was stopped by addition of 0.1 ml 10% perchloric acid. Tubes were chilled, and 1M $KHCO_3$ was added to give a final pH of 6.5. Tubes were centrifuged and heated supernatant fractions were analyzed for C^{14} content.

TABLE II

Comparison of (+)-Palmitylcarnitine and (-)-Palmitylcarnitine on Acetyl CoA Carboxylase Activity in the Presence and Absence of Citrate

Citrate Concentration (mM)	CPM in Malonyl CoA** Control	(+)-Palmitylcarnitine (0.05 mM)	(-)-Palmitylcarnitine (0.05 mM)
0	700	1670	930
18	2700	3950	3750

**Conditions were the same as those reported for Table I, except that all tubes were incubated for 30 minutes at 30°C after addition of 0.4 mg acetyl CoA carboxylase as the last component.

Interactions of citrate, palmitylcarnitine and palmityl CoA with acetyl CoA carboxylase are currently being more thoroughly investigated. Palmityl CoA inhibition of carboxylase activity was partially relieved by (+)-palmitylcarnitine and to a lesser extent by (-)-palmitylcarnitine (Table III). (-)-Palmitylcarnitine may be less effective because of contamination of our acetyl CoA carboxylase preparation with carnitine palmityltransferase and acetyl CoA hydrolase.

TABLE III

Relief of Palmityl CoA Inhibition of Acetyl CoA Carboxylase by (+)- and (-)-Palmitylcarnitine in the Presence and Absence of Citrate and Malonate

Components Added to Basic Medium			CPM in Malonyl CoA***		
Palmityl CoA (mM)	Citrate (mM)	Malonate (mM)	Control	(+)-Palmityl-carnitine (0.042 mM)	(-)-Palmityl-carnitine (0.042)
0	0	0	353	661	471
0.021	0	0	180	382	218
0	1.7	0	845	1072	1055
0.021	1.7	0	571	791	732
0	0	1.7	575	1034	796
0.021	0	1.7	294	547	349

***Conditions were the same as those reported for Table I, except that in all tubes, buffer, citrate, malonate, palmityl CoA and palmitylcarnitine in combinations listed were preincubated with 0.4 mg acetyl CoA carboxylase for 15 minutes before addition of substrates.

Acetyl CoA carboxylase activity ordinarily limits the rate of fatty acid synthesis (Ganguly, 1960; Numa et al, 1961), and it is known that this reaction is inhibited by palmityl CoA (Bortz and Lynen, 1963; Numa et al, 1965). It is therefore tempting to suggest that since (-)-palmitylcarnitine can activate partially purified acetyl CoA carboxylase (Tables II and III), regulation of intracellular levels of (-)-palmitylcarnitine may serve a physiological role in the regulation of the rates of fatty acid synthesis. The ratio of palmityl CoA to palmitylcarnitine in the vicinity of the fatty acid synthesizing system might be critical. The absence of a stimulatory effect by (-)-palmitylcarnitine on fatty acid synthesis in liver supernatant fractions (Figs. 1 and 2) could be interpreted on the basis that palmityl CoA was being generated, thereby antagonizing stimulatory effects of palmitylcarnitine on acetyl CoA carboxylase. (+)-Palmitylcarnitine, however, is an unacceptable substrate for the carnitine palmityltransferase reaction, and consequently its activation of the carboxylase would be unopposed by palmityl CoA. We do not know the basis for the relatively slight stimulation of fatty acid synthesis induced by free carnitine in our preparations as opposed to the large enhancement reported by Kipnis and Kalkhoff (1965). Since different systems were employed, it will be of interest to examine the status of carnitine palmityltransferase activities in the two types of preparations.

SUMMARY

Depressed rates of fatty acid synthesis from acetate-1-C^{14} by liver preparations from starved rats were increased approximately 13-fold by addition of (+)-palmitylcarnitine but not by (-)-palmitylcarnitine. In contrast, malonyl-2-C^{14} CoA incorporation into fatty acids was not decreased by starvation, and it was increased only 30% by (+)-palmitylcarnitine. Partially purified acetyl CoA carboxylase activity was enhanced by either (+)- or (-)-palmitylcarnitine in both the presence and absence of citrate or malonate. Palmityl CoA inhibition could be partially overcome by (+)- or (-)-palmitylcarnitine addition to the isolated enzyme system. A possible role of palmitylcarnitine in the regulation of fatty acid synthesis was discussed.

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