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STUDIES ON THE MECHANISM OF ACTION OF THE HYPOGLYCEMIC AGENT, 2-(3-METHYLCINNAMYLHYDRAZONO)-PROPIONATE (BM 42.304)

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Abstract—A new hypoglycemic agent, 2-(3-methylcinnamylhydrazono)-propionate MCHP (BM 42.304) was shown to be an inhibitor of the transfer of long-chain fatty acids across the mitochondrial inner membrane. The following data support this conclusion: the drug, at already 5 μM, inhibited ketogenesis from oleate but not from octanoate in the perfused guinea-pig liver; likewise, ketogenesis from L-(−)-palmitoylcarnitine and palmitoyl-CoA + L-(−)-carnitine, but not from octanoate, was depressed in isolated guinea-pig liver mitochondria. Oxigraphic measurements of the oxygen uptake by isolated mitochondria showed that the drug impaired oxygen uptake with the long-chain fatty acid derivatives but not with octanoate. Finally, *in vivo* effects of the drug such as hypoketonemia and an increased concentration of free fatty acids in blood are in agreement with the above formulated mechanism of action. A comment is given on the relationships between fatty acid oxidation and gluconeogenesis in the guinea-pig liver.

The observation by Haeckel and Oellerich [1] that phenylethylhydrazono propionate, a derivative of phenylethylhydrazine (phenelzine, an antidepressant drug), exerts an inhibitory effect on gluconeogenesis in the perfused guinea-pig liver led to the description of hydrazono propionate derivatives as a new class of hypoglycemic agents [2–4]. In spite of a detailed investigation of two compounds belonging to this class, i.e. 2-(phenylethylhydrazono)-propionate and 2-(2-cyclohexylethylhydrazono)-propionate with respect to their hypoglycemic action and inhibitory effect on hepatic gluconeogenesis [2–4], their mechanism of action remains still obscure.

Another compound of the above mentioned class of hypoglycemic agents, i.e. 2-(3-methylcinnamylhydrazono)-propionate (BM 42.304, abbreviated here as MCHP) was synthesized and investigated in our laboratories. We have shown that MCHP exhibits an efficient hypoglycemic effect in fasted guinea pigs and that it inhibits gluconeogenesis from a variety of precursors in the perfused guinea-pig liver [5].

It is the purpose of this paper to present experimental data concerning the mechanism of action of MCHP. They suggest that MCHP is an effective inhibitor of the transfer of long-chain fatty acids from cytosol through the mitochondrial inner membrane, impairing their oxidation with the corresponding consequences for the rate of gluconeogenesis.

MATERIALS AND METHODS

Animals

Guinea pigs, mixed strain, of both sexes, weighing 300–400 g, maintained on a laboratory standard diet were used in all experiments. When used as liver donors for either perfusion experiments or for mitochondrial preparation, the animals were starved for 48 hr with free access to water.

Chemicals

MCHP and 3-methylcinnamylhydrazine were synthesized by Drs M. Hübner and R. Heerdt of the Division of Chemistry of Boehringer Mannheim GmbH. Several other chemicals were from the following sources: Na-oleate and L-(−)-carnitine chloride, from Sigma Chem. Co., St Louis, MO, U.S.A.; L-(−)-palmitoyl carnitine and bovine serum albumin (with low content of free fatty acids, see Serva catalog No 11920), from Serva, Heidelberg; palmitoyl-SCoA from either Sigma or Boehringer Mannheim GmbH. All other chemicals, including enzymes and coenzymes (most of them from Boehringer Mannheim GmbH) were of the highest purity commercially available.

In vivo *experiments*

Guinea pigs were given daily, for a period of 6 days, 5 mg/kg MCHP by intragastric intubation; the corresponding controls received daily the same volume of NaCl (0.9%). Twenty-four hours before the last intubation, two groups of animals, each of 10 individuals, i.e. a control group and one treated with MCHP, were deprived of food. These are referred to in Table 4 as "fasted control" and "fasted treated" animals. Three hours after the last intubation the animals were narcotized with Nembutal (5 mg/100 g), the abdomen opened and 3 ml of blood were withdrawn from the exposed inferior vena cava, with the aid of a heparinized syringe. Other two groups of animals, each of 10 individuals, were treated as above with the exception that they were allowed free access to food till the moment of narcosis. These are referred to in Table 4 as "fed control" and "fed treated" animals.

In vitro *experiments*

Liver perfusion. Non-recirculating, hemoglobin-free perfusion of liver was performed as referred to in detail in a previous paper [6]. The perfusion medium, Krebs–Ringer bicarbonate buffer, continuously gassed with $O_2 : CO_2$ (19 : 1), was pumped into the liver through the portal vein at a rate of 45 ml/min. Octanoate, Na-salt, was dissolved in Krebs–Ringer bicarbonate buffer without Ca^{2+} and Mg^{2+} and infused into the perfusion system through an infusion chamber, with the aid of an infusion pump at a rate of 0.20 ml/min; oleate, Na-salt, was first complexed to bovine serum albumin (dialysed but not defatted, see Chemicals) in Krebs–Ringer bicarbonate buffer as for octanoate and given to the liver as above at a rate of 1.5 ml/min to reach the desired final concentration in the perfusion medium (see the corresponding graphs).

Mitochondrial experiments. Mitochondria were isolated as described by Barzu *et al.* [7] in a medium containing (mM final concentration): sucrose, 250; triethanolamine, 10 and K-EDTA, 0.1, at pH 7.45. The respiratory control index of usually three-times washed mitochondria ranged between 5 and 9 with glutamate and malate as respiratory substrates. For studies on the rate of phosphoenolpyruvate (PEP) synthesis or ketone body formation, mitochondria were incubated in a basal medium containing (mM, final concentration): sucrose, 190; KCl, 15; $MgCl_2$, 5; K-EDTA, 1; triethanolamine.HCl, 50; K-phosphate, 10; ADP, 2; bovine serum albumin (dialysed but not defatted, see Chemicals), 0.5 mg/ml; the final pH was adjusted to 7.45. Any other additions to the incubation medium were done after previous adjustment of the solutions to pH 7.45. Mitochondrial protein ranged between 4 and 6 mg/ml incubation medium. The incubation was carried out in a final volume of 4 ml for 20 min (ketone body experiments) or 30 min (PEP formation), at 37°, on a shaking water bath (120 strokes/min), with oxygen as gaseous phase, in 25 ml Erlenmeyer flasks. At the intervals indicated in the corresponding tables and graphs, 1 ml of incubation medium was collected and treated with 0.1 ml perchloric acid (70%, w/v); after centrifugation and neutralization (at 2–4°) with KOH in the presence of triethanolamine (50 mM, final concentration) the samples were immediately used for ketone body or PEP assay (see below). Isolated mitochondria were also used in oxigraphic experiments to test the effect of MCHP and its corresponding hydrazine on the oxygen uptake in the presence of different substrates. The basal medium used in this series of experiments consisted of (mM, final concentration): KCl, 120; $MgCl_2$, 5; triethanolamine.HCl, 20; KH_2PO_4, 10; K-EDTA, 0.5; L-malate, 0.5; bovine serum albumin (dialysed but not defatted, see above), 0.5 mg/ml. Mitochondrial were added at a concentration of 0.8–1.2 mg/ml of reaction mixture. Other additions are as described in the corresponding tables and graphs.

Metabolite assay

Acetoacetate [8], 3-hydroxybutyrate [9], glucose [10], non-esterified fatty acids [11] and triglyceride [12], were analysed according to the cited procedures. Oxygen consumption by isolated mitochondria was measured polarographically using a Clark type oxygen electrode (Echweil, Kiel, F.R.G.) attached to a polarograph (Transdyne General, Ann Arbor, MI, U.S.A.) and to a potentiometric recorder.

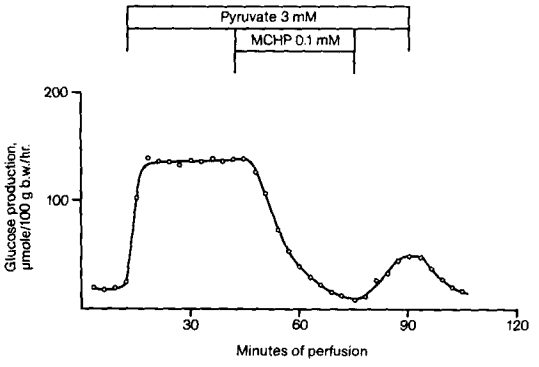

Fig. 1. Effect of MCHP on the rate of glucose formation from pyruvate in the perfused guinea-pig liver. The horizontal columns on this and subsequent graphs representing perfusion experiments (i.e. the graphs in Figs. 2–5) show the period of time for which a compound (mentioned inside the columns) was being infused into the perfusate to give the final concentration specified inside the columns. The plotted points on this graph and on those in Figs. 2 and 3 represent the mean value for at least two perfusions; in Figs. 4 and 5 at least 5 livers were taken in each experimental variant. Figs. 2–4 are self-explanatory.

RESULTS

Effect of octanoate and of oleate on the inhibitory action of MCHP on gluconeogenesis in the perfused liver

The inhibitory effect of MCHP on the glucose formation from pyruvate in the perfused liver (Fig. 1) was no longer seen in the presence of octanoate (Fig. 2). When, on the other hand, the fatty acid in the perfusion medium was oleate, MCHP continued to exert its inhibitory effect on glucose formation from pyruvate (Fig. 3). These results constituted the starting point for a series of experiments in which the effect of MCHP was studied on the rate of ketogenesis.

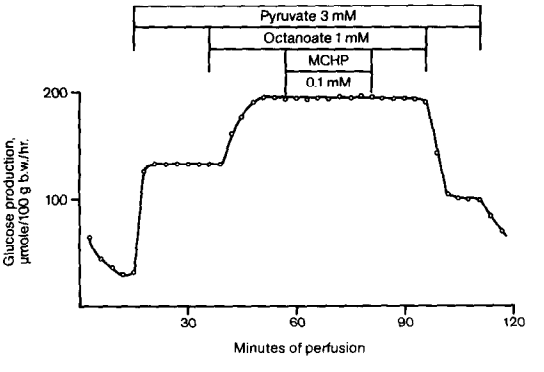

Fig. 2.

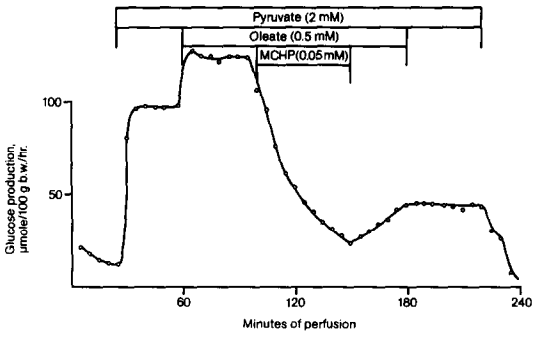

Fig. 3.

Effect of MCHP on the rate of ketogenesis with octanoate and oleate in the perfused liver

While the rate of ketogenesis from octanoate (Fig. 4) was not affected by MCHP at a concentration (0.05 mM) high enough to produce an almost total inhibition of glucose formation in the perfused liver [5], ketogenesis from oleate was strongly inhibited by MCHP (Fig. 5). A concentration as low as 0.005 mM of MCHP produced already an inhibition of about 50% of the rate of ketogenesis (Fig. 5). A slight tendency towards recovery of the rate of ketogenesis was observed after withdrawal of the inhibitor from the perfusion medium (Fig. 5).

Effect of MCHP on the rate of ketogenesis by isolated mitochondria

As expected on the basis of the results obtained on perfused liver, MCHP did not affect the rate of ketogenesis with octanoate (Fig. 6) but inhibited this process when precursors of ketone bodies were long-chain fatty acid derivatives, i.e. L-(−)-palmitoylcarnitine (Fig. 7) and palmitoyl-CoA + L-(−)-carnitine (Fig. 8). The concentration of MCHP that produced a 50% inhibition of the rate of ketogenesis in isolated mitochondria was higher than that in the perfused liver (i.e. 20 μM with L-(−)-palmitoylcarnitine, Fig. 7, and between 50 and 100 μM with palmitoyl-CoA + L-(−)-carnitine, Fig. 8). It is also noteworthy that the rate of ketogenesis with only palmitoyl-CoA, i.e. without L-(−)-carnitine, was

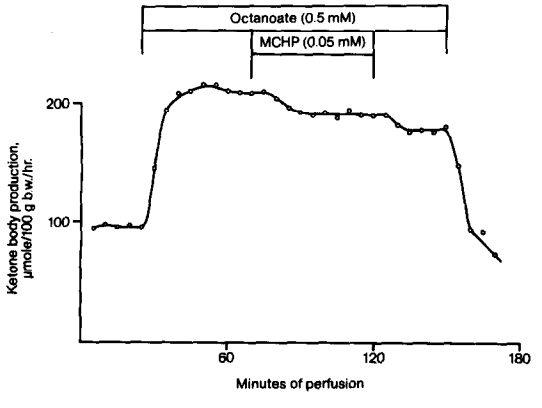

Fig. 4.

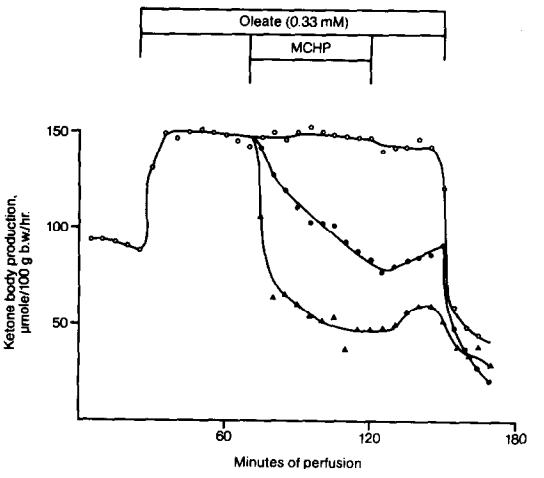

Fig. 5. Effect of MCHP on the rate of ketogenesis from oleate by the perfused guinea-pig liver. ○, Control; ●, MCHP, 0.005 mM; △, MCHP, 0.05 mM.

almost the same as the rate of endogenous ketogenesis (Fig. 8). This is also an indication on the degree of intactness of mitochondrial inner membrane.

Effect of MCHP on the oxygen uptake by isolated mitochondria in the presence of various substrates

When the respiratory substrates were either succinate (Table 1) or glutamate + malate (table 2), MCHP did not affect the rate of oxygen consumption even at concentrations exceedingly higher (0.5 mM) than those necessary to produce a total inhibition of either gluconeogenesis [5] or ketogenesis (Figs 5, 7 and 8, this paper). When long-chain fatty acid

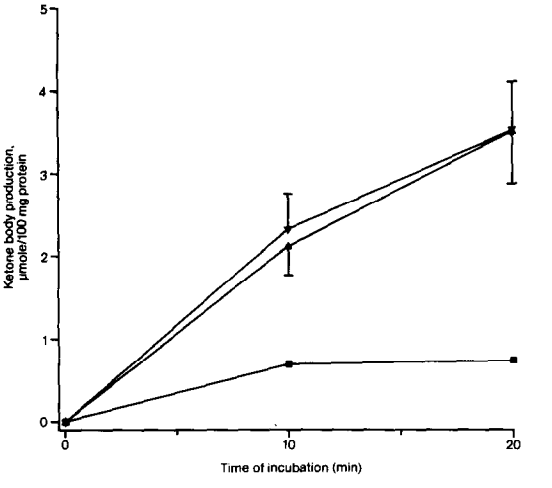

Fig. 6. Effect of MCHP on the rate of ketogenesis from octanoate by isolated guinea-pig liver mitochondria. ■, Endogenous ketogenesis; ▲, Octanoate, 0.5 mM; ▼, Octanoate + MCHP (0.1 mM). The vertical bars are SEM for at least 4 mitochondrial preparations. Where the vertical bars are not drawn, the SEM was too small to be represented at the scale. Other details are given under Materials and Methods section.

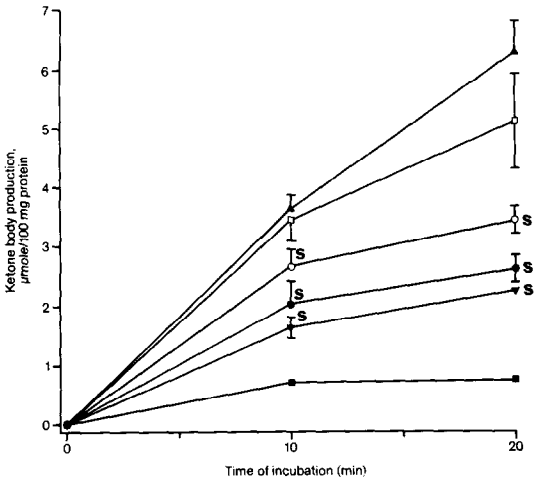

Fig. 7. Effect of various concentrations of MCHP on the rate of ketogenesis from L-(−)-palmitoylcarnitine by isolated guinea-pig liver mitochondria. ■, Endogenous ketogenesis ($n = 9$); ▲, L-(−)-palmitoylcarnitine (PC, 0.2 mM, $n = 9$). □, PC (0.2 mM) + MCHP (0.01 mM) ($n = 4$); ○, PC (0.2 mM) + MCHP (0.02 mM) ($n = 5$); ●, PC (0.2 mM) + MCHP (0.05 mM) ($n = 6$); ▼, PC (0.2 mM) + MCHP (0.10 mM) ($n = 6$). Vertical bars, as in Fig. 1. The points marked with "s" are at a statistically significant difference ($P < 0.05$ or smaller) from the samples incubated without inhibitor. Other details are given in Materials and Methods section.

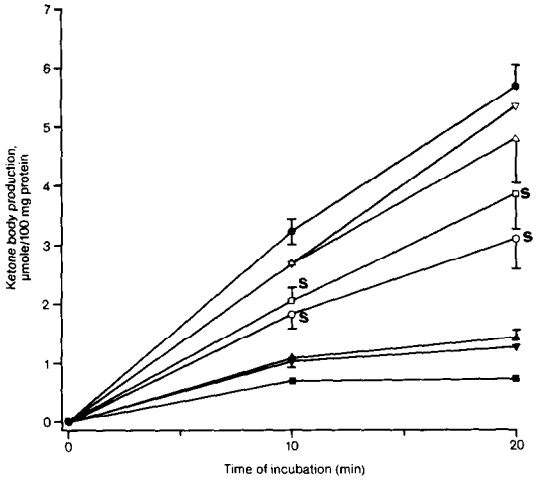

Fig. 8. Effect of various concentrations of MCHP on the rate of ketogenesis from palmitoyl-CoA and L-(−)-carnitine by isolated guinea-pig liver mitochondria. ■, Endogenous ketogenesis; ▲, Palmitoyl-CoA (PCoA. 0.2 mM, $n = 7$); ▼, PCoA (0.2 mM) + MCHP (0.1 mM, $n = 3$); ●, PCoA (0.2 mM) + L-(−)-carnitine (LC, 0.1 mM, $n = 9$); ▽, PCoA (0.2 mM) + LC (0.1 mM) + MCHP (0.01 mM, $n = 3$); △, PCoA (0.2 mM) + LC (0.1 mM) + MCHP (0.02 mM, $n = 4$); □, PCoA (0.2 mM) + LC (0.1 mM) + MCHP (0.05 mM, $n = 4$). O, PCoA (0.2 mM) + LC (0.1 mM) + MCHP (0.1 mM, $n = 4$); vertical bars and "s" as in Fig. 7. Other details are given under the Materials and Methods section.

Table 1. Effect of MCHP on the respiratory control index (RCI) and on ADP/O ratio in isolated guinea-pig liver mitochondria with succinate (10 mM) as respiratory substrate

Conditions	Oxygen uptake (ngatoms/min/mg protein) State 3	State 4	RCI	ADP/O
Control				
1st ADP addition	130.6	26.1	5.00	1.77
2nd ADP addition	158.7	26.6	5.96	
3rd ADP addition	161.3	25.6	6.30	
4th ADP addition	163.8	20.5	8.00	1.58
MCHP (0.5 mM)				
1st ADP addition	143.4	31.7	4.52	1.64
2nd ADP addition	153.6	30.2	5.08	
3rd ADP addition	168.9	26.6	6.35	
4th ADP addition	143.4	23.0	6.22	1.60

ADP was added in four sequences so as to reach, after each addition, a final concentration of 0.15 mM. Other details are given under Materials and Methods.

derivatives such as L-(−)-palmitoyl carnitine (Fig. 9) or palmitoyl-CoA + L-(−)-carnitine (Fig. 10) served as respiratory substrates, a clear cut inhibition of the oxygen uptake by isolated mitochondria was noticed in the presence of different concentrations of MCHP. As expected on the basis of previous experiments, no effect of MCHP was noticed on the mitochondrial oxygen uptake in the presence of octanoate. The corresponding hydrazine derivative of MCHP, i.e. 3-methylcinnamylhydrazine (0.1 mM) had no effect on mitochondrial oxygen uptake in the presence of either substrate (not shown here).

Effect of MCHP on PEP synthesis by isolated mitochondria

Since PEP synthesis in guinea-pig liver is to a great extent an intramitochondrial process [13], it was of interest to test the effect of MCHP on the rate of formation of this key metabolite in gluconeogenesis.

Table 2. Effect of MCHP on the respiratory control index (RCI) and on ADP/O ratio in isolated guinea-pig liver mitochondria with glutamate (10 mM) and malate (10 mM) as respiratory substrates

Conditions	Oxygen uptake (ngatoms/min/mg protein) State 3	State 4	RCI	ADP/O
Control				
1st ADP addition	73.7	12.6	5.85	2.40
2nd ADP addition	90.6	13.8	6.55	
3rd ADP addition	92.1	14.1	6.52	
4th ADP addition	95.2	13.5	7.04	2.39
MCHP (0.5 mM)				
1st ADP addition	79.9	15.9	5.00	2.38
2nd ADP addition	95.2	16.6	5.74	
3rd ADP addition	90.6	15.9	5.67	
4th ADP addition	92.1	13.8	6.67	2.35

ADP additions as in Table 1. Other details are given under Materials and Methods.

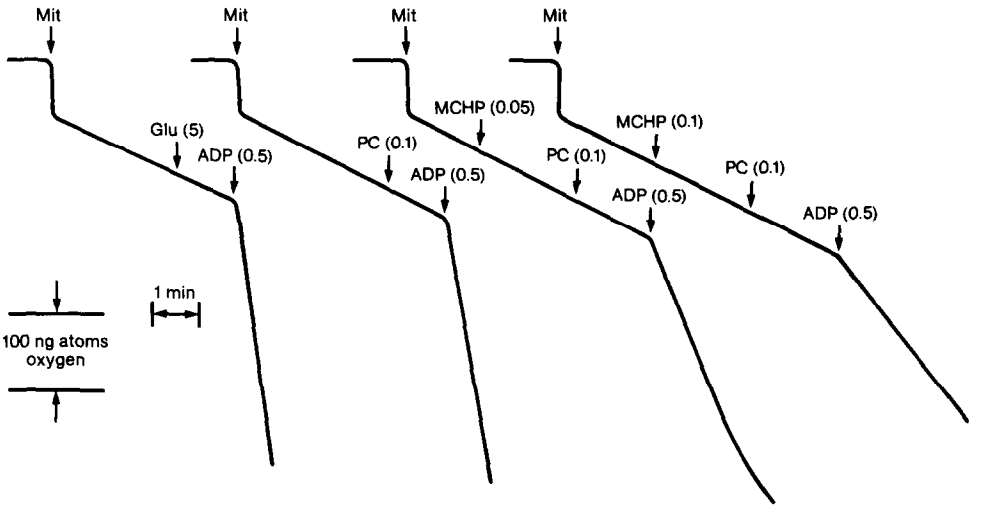

Fig. 9. Typical recorder traces showing the effect of MCHP on the rate of oxygen uptake by isolated guinea-pig liver mitochondria in the presence of glutamate (Glu) or L-(−)-palmitoylcarnitine (PC). The arrows show the moment of addition of the specified ingredient to the oxigraphic chamber. The figures in parentheses show the final concentration of the added compound. Mit, mitochondria. Other details are given under the Materials and Methods section.

As shown in Table 3, MCHP, at concentrations much higher (0.5 mM) than necessary to produce an inhibition of gluconeogenesis [5] or of ketogenesis (this paper), did not affect the rate of PEP formation.

Effect of MCHP on a few biochemical parameters in guinea pig in vivo

As one might have expected from the *in vitro* results, MCHP produced changes *in vivo* in agreement with the supposition that its mechanism of action consists in impairing the oxidation of long-chain fatty acids. Thus, beside the hypoglycemia, an increase in the concentration of non-esterified fatty acids associated with a decrease of the ketone body concentration in blood were evident (Table 4). No significant changes of triglyceride concentration in blood were noticed.

DISCUSSION

The experimental data presented in this paper allow the supposition that MCHP is an inhibitor of the transfer of long-chain fatty acids from cytosol, through mitochondrial inner membrane, to the site of their oxidation. This emerges from the fact that MCHP does not affect the rate of either ketogenesis, both in perfused liver and isolated mitochondria, or of oxygen uptake by isolated mitochondria with octanoate as substrate; when, however, the substrates were long-chain fatty acids (oleate in perfusion experiments) or corresponding derivatives (i.e.

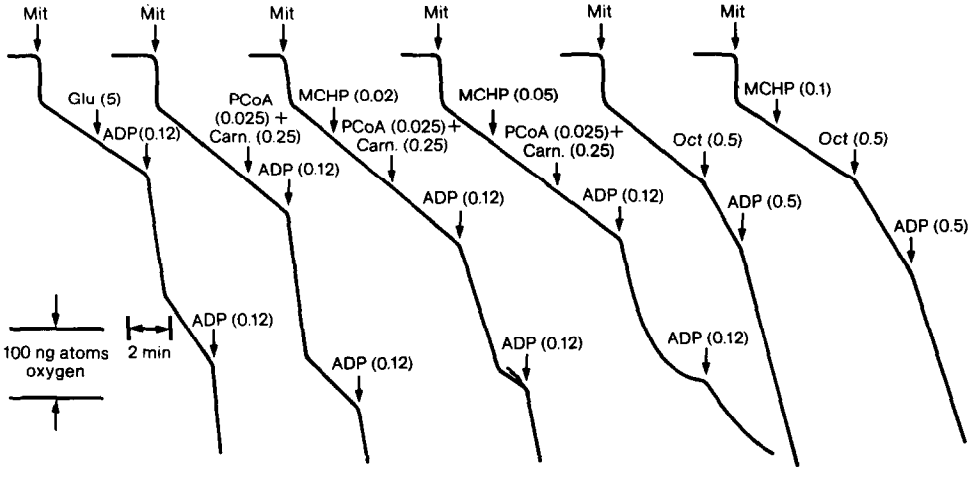

Fig. 10. Typical recorder traces showing the effect of MCHP on the rate of oxygen uptake by isolated guinea-pig liver mitochondria in the presence of palmitoyl-CoA (PCoA) + L-(−)-carnitine (Carn) or octanoate (Oct). The significance of the arrows and the figures in parentheses, as in Fig. 9. Other details are given in the Materials and Methods section.

Table 3. Effect of MCHP on the rate of phosphoenolpyruvate (PEP) synthesis by isolated guinea-pig liver mitochondria

Additions (mM)	PEP formed (μmol/10 mg prot./30 min)
2-Oxoglutarate (10)	1.31
2-Oxoglutarate (10) + MCHP (0.5)	1.42
Fumarate (10)	2.31
Fumarate (10) + MCHP (0.5)	2.32
Succinate (10)	0.98
Succinate (10) + MCHP (0.5)	1.19
Malate (10)	2.08
Malate (10) + MCHP (0.5)	2.18

Results in the table are taken from a representative experiment. Details are given under Materials and Methods.

L-(−)-palmitoylcarnitine and palmitoyl-CoA + L-(−)-carnitine in mitochondrial experiments) both ketogenesis and oxygen uptake were inhibited by the compound.

It was long ago established that octanoate transport from cytosol into mitochondria does not require the participation of carnitine [14]. On the other hand, it is classically recognized that the reactions in the sequence of β-oxidation itself are identical for different fatty acids, no matter how long is their chain. Therefore, the only difference between octanoate and long-chain fatty acids such as oleate or palmitate with respect to their oxidative metabolism is the requirement of a special mechanism for the transport of long-chain fatty acids across the mitochondrial inner membrane. Consequently, our supposition that this is the step at which MCHP affects fatty acid oxidation appears reasonable.

It was already documented that the transport mechanism of long-chain fatty acids across the mitochondrial membrane is effected by two distinct transferases: carnitine acyltransferase I and II (EC 2.3.1.21) [15–18]. Moreover, it has been shown that the flux of fatty acids through these transferases is the rate limiting and, therefore, the regulatory step of ketogenesis [19, 20]. As a consequence, any changes of the activity of these enzymes will affect the rate of ketogenesis in either perfused liver or isolated mitochondria.

By using two different derivatives of fatty acids, i.e. L-(−)-palmitoylcarnitine and palmitoyl-CoA + L-(−)-carnitine, we have tried to establish which of these two enzymes is inhibited. Since the ketogenesis and the oxygen uptake by isolated mitochondria were inhibited by MCHP with both long-chain fatty acyls, we concluded that both transferases are affected by MCHP. It seems, however, that the inner enzyme, i.e. carnitine acyltransferase II, is more sensitive to the inhibition by MCHP since both ketogenesis and oxygen uptake by isolated mitochondria were inhibited to a higher extent with L-(−)-palmitoyl carnitine as the substrate. In this respect MCHP differs from 2-tetradecylglycidic acid (McN-8302) which has been shown to inhibit only the so-called "overt" enzyme or carnitine acyltransferase I [21]. Work is in progress in our laboratory to see whether the activity of these enzymes is inhibited only when they are incorporated within the mitochondrial inner membrane or, also, when they are released from this structure.

The *in vivo* effects of MCHP on a few biochemical parameters in the guinea pigs, as reported in this paper, are in agreement with the inhibition of fatty acid oxidation. From this point of view our compound resembles the *in vivo* effects of tetradecylglycidic acid [22] as well as of other recently reported hypoglycemic agents — phenylalkyloxirane carboxylic acids [23]. More experimental data will be presented elsewhere about the effect of MCHP on the metabolism of different organs and tissues.

A comment should be given here on the relationship between fatty acid oxidation and gluconeo-

Table 4. Effect of MCHP on the concentration of ketone bodies (AcAc, acetoacetate, and 3-OHB, 3-hydroxybutyrate) in the whole blood and of free fatty acids (FFA) and triglycerides (TG) in blood serum of guinea pigs

Conditions	Ketone bodies		FFA	TG
	AcAc	30HB		
	(μM)		(mM)	(mg/100 ml)
Fed control	36.4 ± 2.9	4.6 ± 1.1	0.10 ± 0.01	53.6 ± 3.9
Fed treated	40.7 ± 4.4	5.1 ± 1.1	0.99 ± 0.16*	72.6 ± 19.6
Fasted control	159.2 ± 24.6	158.8 ± 31.3	0.95 ± 0.13	124.7 ± 30.1
Fasted treated	69.1 ± 7.2*	38.1 ± 14.9*	3.62 ± 0.26*	83.5 ± 10.6

The values are mean ± SEM for 8–10 animals in each group.

* Statistically significant difference towards the corresponding control at a $P < 0.01$. Other details are given under Materials and Methods.

genesis in guinea-pig liver. This seems to be justified when one has to do with a compound such as MCHP, whose inhibitory effect on gluconeogenesis is coincident with an impairment of the transfer of long-chain fatty acids across the mitochondrial inner membrane.

The inhibitory effect of fatty acids on gluconeogenesis from lactate in the perfused guinea-pig liver, discovered by Söling *et al.* [24], does not seem to reflect an *in vivo* situation. Such an effect is restrained to the liver under the conditions of perfusion and is due, no doubt, mainly to the displacement of the mitochondrial redox state to a more negative value as a consequence of fatty acid oxidation [25, 26]. This is also clearly demonstrated by the data of Jomain-Baum and Hanson [27] showing that the inhibitory effect of fatty acids on glucose formation in the perfused liver is counteracted by NH_4Cl which acts as a mitochondrial oxidant by the virtue of its participation in the glutamate dehydrogenase reaction. One should not disconsider that ammonia is permanently supplied to the liver through the portal vein, under the *in vivo* conditions, at concentrations of about 1 mM [27], sufficient to buffer the tendency towards a drastical displacement of the mitochondrial redox state produced when only fatty acids would be supplied to the liver. In confirmation of this point of view is the stimulatory effect of both octanoate and oleate on gluconeogenesis from pyruvate, a glucose precursor more oxidized than the glucose itself (this paper). Such a stimulation is due to the same mechanism which is also responsible for inhibition of gluconeogenesis from lactate, i.e. the displacement of the mitochondrial redox state to a more negative value. When pyruvate is the glucose precursor, however, such a displacement will favour the rate of glucose formation.

In vivo the liver receives a mixture of lactate + pyruvate at a ratio of about 10 and also other precursors whose conversion into glucose requires energy (ATP), reducing power (NADH) and enzyme efectors (acetyl-CoA). No other metabolic pathway than β-oxidation in cooperation with the citric acid cycle would fulfil better such requirements. Therefore, an inhibition of entry of long-chain fatty acids into their oxidative pathway, as it happens in the presence of MCHP and 2-tetradecylglycidic acid, is expected to induce an impairment of gluconeogenesis. Such an impairment should be seen not only with three-carbon precursors (e.g. lactate, pyruvate, alanine) but also with others that enter the gluconeogenic pathway beyond the level of pyruvate carboxylase (e.g. propionate, glutamine, glycerol, fructose). This was the case with both tetradecylglycidic acid [28] and with MCHP [5]. One could, however, expect that the rate of gluconeogenesis from precursors such as glycerol and fructose is inhibited to a lower extent than with precursors such as lactate and pyruvate. This was the case, too [5].

Finally, a word should be said about the possible difference with respect to the mechanism of action between hydrazine, such as phenylethylhydrazine and MCHP. It has been shown that phenylethylhydrazine [29] and its corresponding hydrazone with pyruvate [30] are inhibitors of glutamate-oxaloacetate and glutamate-pyruvate transaminases and that this might account at least in part for their inhibitory effects on gluconeogenesis. Moreover, it has been suggested that the hydrazono derivatives of pyruvate, such as phenylethylhadrazono propionate are first hydrolytically converted to pyruvate and corresponding hydrazine [30]; the last might be, as mentioned above, the "true" inhibitor. The data presented in this paper do not allow an extension of the mechanism of action as suggested for MCHP to other structurally related compounds such as phenylethylhydrazono and 2-(2-cyclohexylethylhydrazono)-propionate nor do they permit the suggestion that MCHP might act as an inhibitor after its hydrolysis to pyruvate and the corresponding hydrazine. As already mentioned in this paper, 3-methylcinnamylydrazine did not mimic the effects of MCHP on the oxygen uptake by isolated liver mitochondria in the presence of various substrates.

Experimental work is in progress in our laboratory to study the intimate mechanism of action of MCHP and also other properties of this inhibitor in view of its usefulness for the treatment of diabetes.

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