Biochemical Pharmacology, Vol. 40, No. 8, pp. 1695–1699, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

ON THE MECHANISM OF SODIUM 2-5-4 CHLOROPHENYLPENTYLOXIRANE-2-CARBOXYLATE (POCA) INHIBITION OF HEPATIC GLUCONEOGENESIS

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(*Received* 6 *July* 1989; *accepted* 12 *May* 1990)

Abstract—Inhibition of hepatic long chain fatty acid oxidation by 2-5-4 chlorophenylpentyloxirane-2-carboxylate (POCA) leads to decreased gluconeogenic rates from lactate or from low concentrations of pyruvate. The inhibitory effect is fully overcome by concentrations of pyruvate above 0.8 mM or by the simultaneous administration of a medium chain fatty acid. At low pyruvate availability the energy cost of gluconeogenesis is mainly supported by fatty acid oxidation and POCA-induced inhibition of glucose production is secondary to a decreased energy availability. This is supported by the following observations: (i) POCA decreases hepatic respiration and phosphorylation potential; (ii) the rate of pyruvate-induced respiration was the same regardless of whether gluconeogenesis was inhibited or not by POCA; and (iii) concentrations of pyruvate above 0.8 mM, at which gluconeogenesis is not inhibited, prevented the POCA-induced decrease in the phosphorylation potential. It is concluded that inhibition of long chain fatty acid oxidation by POCA leads to a switch of energy fuel, and results in the oxidation of more pyruvate to meet the cellular energy demands. When pyruvate availability is low and thus, presumably, its mitochondrial transport restricted, pyruvate carboxylation most probably becomes limiting as a result of the increased flux through pyruvate dehydrogenase, in the presence of POCA.

Fatty acids are well known modulators of the hepatic gluconeogenic pathway [1–3]. The role of fatty acids in the control of gluconeogenesis is supported by the following evidence: firstly, physiological or pathological conditions accompanied by hyperlipemia, like starvation or diabetes, are characterized by increased rates of hepatic glucose output [4–6]. Secondly, indirect evidence indicates that oxidation of fatty acids varies in parallel to the rate changes of gluconeogenesis [7]; and, finally, inhibitors of long chain fatty acid oxidation have been shown to inhibit glucose production [8] by a mechanism not yet clarified. The kinetics of fatty acid interaction with the gluconeogenic pathway and their mechanism of action have been recently described in detail [9]. According to that study, exogenous fatty acids appear to increase glucose production by enhancing pyruvate transport across the mitochondrial membrane [9]. However, other possible roles, for example fatty acid acting as an energy fuel, cannot be ruled out in view of the fact that a normal rate of long chain fatty acid oxidation is required to maintain adequate glucose production. The present investigation was undertaken to elucidate the correlation existing between the action of the hypoglycemic agent 2-5-4 chlorophenylpentyloxirane-2-carboxylate (POCA) in perturbing cellular energy production and its capacity to inhibit gluconeogenesis [10, 11]. POCA belongs to the class of oxirane carboxylic acids whose mechanism of action in inhibiting long chain fatty acid oxidation is related to their ability to inactivate carnitine palmitoyl transferase I and therefore the mitochondrial transport of fatty acids [12].

Our results indicate that the starved liver relies largely on fatty acid as an energy fuel. The action of POCA in inhibiting gluconeogenesis can be explained on the basis of its capacity to decrease the cellular energy state by preventing long chain fatty acid oxidation.

MATERIAL AND METHODS

Materials. Sodium pyruvate and octanoate that were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). All other reagents were obtained from Merck (Darmstadt, F.R.G.). The enzymes used for metabolites determination [lactate dehydrogenase (EC 1.1.1.27), glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and hexokinase (EC 2.7.1.1)] were purchased from Boehringer (Mannheim, F.R.G.). 2-5-4 Chlorophenylpentyloxirane-2-carboxylate (POCA) was kindly donated by Byk Gulden Pharmazeutika (Konstantz, F.R.G.).

Experimental procedures. Livers isolated from 48 hr starved male rats, of the Wistar strain, 180–200 g in body weight, 6 weeks old, were perfused in a non-recirculating perfusion system with Krebs–Ringer bicarbonate buffer at 36.5°. The animals were kept under controlled conditions of light and temperature. They were fed *ad lib.* on a standard laboratory diet and starved for 48 hr prior to their experimental use. The pH of the perfusate medium was 7.4 after equilibration with a gas mixture of 95% O_2: 5% CO_2. The perfusion flow rate rate was 28 ± 2 mL/min. Perfusate pO_2 was continuously monitored with a Clark type oxygen electrode. Substrates were administered diluted in the buffer. Linear increases in substrate supply were attained by

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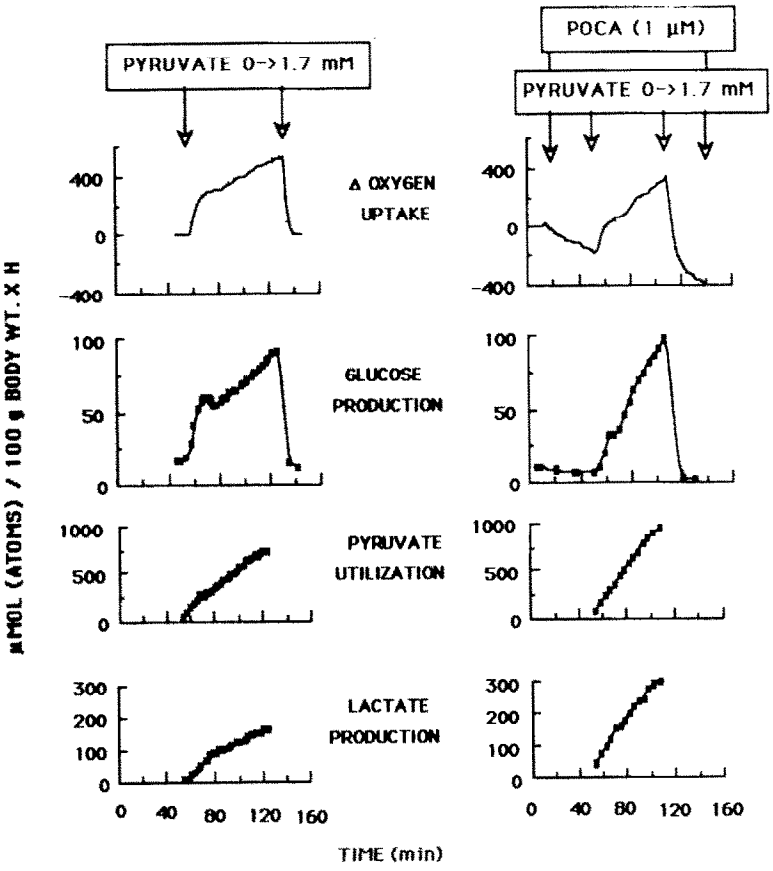

Fig. 1. Effect of 2-5-4 chlorophenylpentyloxirane-2-carboxylate (POCA) on hepatic pyruvate metabolism. Livers from 48 hr starved rats were perfused in a non-recirculating system with Krebs–Ringer bicarbonate buffer (KRB) at a flow rate of 28–32 mL/min. The inflow perfusate temperature was 36.5°, and the pH 7.4 after equilibration of the perfusion medium with a 95.5% CO_2/O_2 mixture. The pO_2 in the outflow perfusate was continuously monitored with a Clark type platinum electrode. At the indicated time pyruvate was administered at a constant increasing rate of 0.016 μmol/min up to a concentration of 1.7 mM. In the right panel the livers were preperfused for 50 min with 1 μM POCA before the pyruvate administration was started. Each experiment was repeated 4–6 times, and representative experiments are shown.

placing the perfusate medium in gradient formers of adequate dimensions. The livers were routinely allowed to equilibrate for 30 min before the experiments were started.

Analytical procedures. Perfusate samples were collected every two min and analysed immediately after the experiment was completed by procedures described previously [13]. Adenine nucleotides were measured in perchloric acid extracts of freeze clamped liver biopsies by high performance liquid chromatography by using a HP 1090 chromatograph and an ionic exchange column APS hypersil (NH_2) (200 × 4.6 mm) from Hewlett–Packard as previously described [14].

RESULTS

Effect of POCA on hepatic respiration and pyruvate metabolism

Figure 1 (left upper panel) shows the effect of linearly increasing concentrations of pyruvate on hepatic gluconeogenesis and oxygen consumption. Steady state production of glucose is observed at a concentration of pyruvate of >3 mM; however, as substrate concentration increased above 0.7 mM, gluconeogenesis increased almost linearly as a function of pyruvate availability (Fig. 2). This kinetic response indicates the existence of two saturable processes in gluconeogenesis from pyruvate. The rates of oxygen utilization followed similar patterns of response to that of glucose production. Assuming a P:O ratio of 3, and an energy cost of 6 moles of ATP per mole of glucose synthesized from pyruvate, stoichiometrically the pyruvate-induced oxygen consumption can be accounted for by the energy demand for the observed glucose production. The utilization of pyruvate was higher than accounted for by glucose and lactate production (Fig. 1, lower left panels). This finding indicates that reactions other than its reduction to lactate or metabolic conversion to glucose account for a significant part of its utilization. The administration of POCA led to a progressive

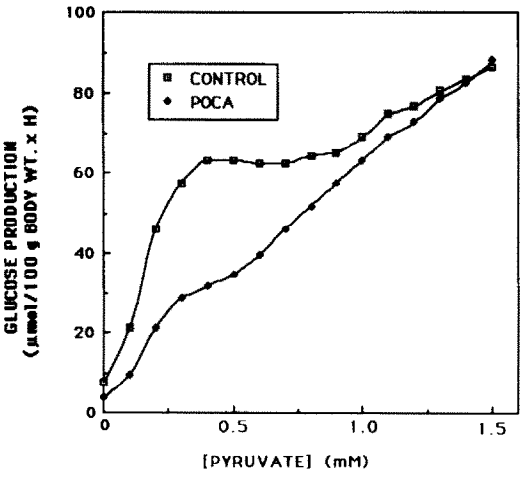

Fig. 2. Effect of 2-5-4 chlorophenylpentyloxirane-2-carboxylate (POCA) on gluconeogenesis from increasing concentrations of pyruvate. The experimental conditions were those described in Fig. 1. Rates of glucose production in the absence (□) as well as in the presence (◆) of 1 μM POCA from experiments shown in Fig. 1, have been combined to facilitate their evaluation. For the sake of clarity intermediate points have been omitted.

decrease in the rate of hepatic respiration (Fig. 1, upper right panel). In view of this observation, it seems plausible to conclude that in starved livers, the basal respiration, and therefore energy production, relies largely on endogenous long chain fatty acid oxidation. In the presence of POCA, the pyruvate to glucose flux was significantly inhibited at concentrations of substrate below 0.7 mM (Fig. 1, right panel and Fig. 2); however, as the substrate availability increased, the rate of gluconeogenesis approached the control values (Fig. 2). Even though POCA reduced the energy demand by decreasing glucose production, pyruvate-induced respiration was not reduced to the same extent (Fig. 1, upper right panel), indicating that more pyruvate is being oxidized to compensate for the inhibition of long chain fatty acid oxidation. The overall rates of pyruvate utilization were not affected by POCA (Fig. 1, right panel). Therefore, the effect of this inhibitor is selectively exerted on energy production and on pyruvate to glucose flux when substrate availability is within the expected physiological range.

Effect of exogenous fatty acids on the POCA-induced perturbation of pyruvate metabolism

Administration of 0.1 mM octanoate to perfused livers induced a steady state increase of respiration (Fig. 3, upper left panel). This finding is consonant with previous reports describing that oxirane carboxylic compounds do not prevent medium chain fatty acid oxidation. In this condition, increasing the supply of pyruvate led to significant increases in the rates of gluconeogenesis and respiration at any concentration of substrate tested (Fig. 3, left panel, and Fig. 4). When 0.1 mM octanoate was administered to livers pretreated with POCA, the rate of respiration was brought up to the basal levels observed prior to POCA treatment (Fig. 3, upper right panel). Despite the presence of POCA, the administration of increasing concentrations of pyruvate led also to increased rates of gluconeogenesis, virtually similar to those observed in the control (Fig. 3, right panel, and Fig. 4). Therefore, the inhibitory action of POCA on gluconeogenesis can be negated either by administering high pyruvate loads or by overcoming the deficiency in energy production with a supply of a medium chain fatty acid whose oxidation is not impeded by POCA.

DISCUSSION

Inhibition of long chain fatty acid oxidation by POCA has been previously reported to perturb gluconeogenesis [15, 16]; however, the mechanism involved has not yet been elucidated. In contrast to previous reports, we observed that concentrations of pyruvate above 1 mM (Figs 1 and 2) completely reversed the inhibitory effect of POCA. When lactate was administered up to a concentration of 10 mM the inhibitory action of POCA was not impeded (results not shown). Regardless of the mechanism by which fatty acid oxidation supports gluconeogenesis, it can be said that it can also be done as effectively by high pyruvate loads. This finding also allows us to conclude that constant infusion of 1 μM POCA do not induce deleterious effects in the hepatic metabolic performance.

The reversibility of the POCA effect by a medium chain fatty acid, like octanoate (Figs 3 and 4) suggest that POCA might inhibit gluconeogenesis by decreasing the hepatic content of acetyl CoA, an obligatory effector for the pyruvate carboxylase reaction [17]. However, this may not be the case since pyruvate prevents the effect of POCA in the absence of detectable changes in the hepatic content of acetyl CoA (results not shown). Pyruvate and/or octanoate both increase the hepatic respiration (Figs 1 and 3) and also the ATP/ADP ratio from 1.5 ± 0.11 after 60 min of treatment with POCA to values (4.8 ± 0.27) approaching those observed under initial basal conditions or in control livers in the absence of POCA. Therefore, the observed inhibition of gluconeogenesis when pyruvate availability is low may be the result of a competition for pyruvate between the mitochondrial dehydrogenase and carboxylase reactions. In the presence of POCA the energy availability is low and the NAD redox potential shifted to a more oxidized state. These conditions would favor pyruvate decarboxylation. That this might be the case is supported by the observation that at concentrations of substrate up to 0.4 mM the rates of pyruvate-induced respiration in the presence of POCA (Fig. 1, right panel) were higher than expected to meet the energy demand for the measured rates of glucose production.

Flux through pyruvate dehydrogenase is saturated at relatively low pyruvate concentrations. Therefore, the obvious question is what a high pyruvate load does to overcome the POCA-induced inhibition of gluconeogenesis. At concentrations of pyruvate above 0.4 mM, its mitochondrial entry occurs mainly by free diffusion [18, 19]. Since the K_m of pyruvate carboxylase for pyruvate is 0.4 mM or higher,

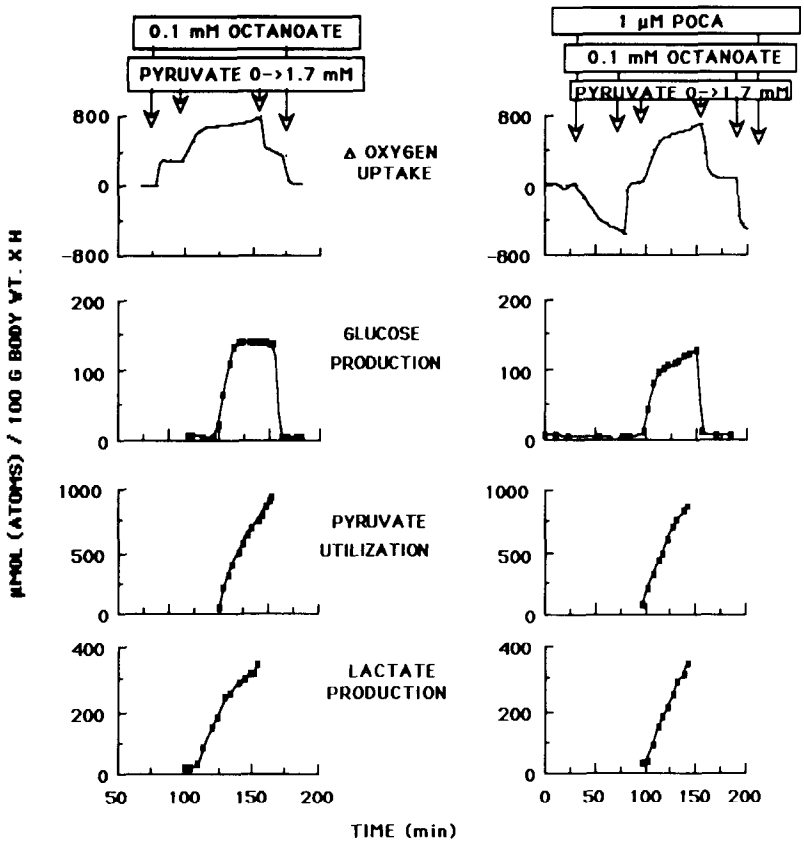

Fig. 3. Effect of octanoate on pyruvate metabolism in control and 2-5-4 chlorophenylpentyloxirane-2-carboxylate (POCA) treated livers. Livers from 48 hr starved rats were perfused in a non-recirculating system with KRB buffer. The experimental conditions were similar to those described in legend to Fig. 1. At the indicated times 1 μM POCA or 0.1 mM octanoate were administered diluted in the buffer. Pyruvate was administered at a constant increasing rate of 0.016 μmol/min as described in Materials and Methods. Each experimental condition has been performed at least four times and representative experiments were plotted.

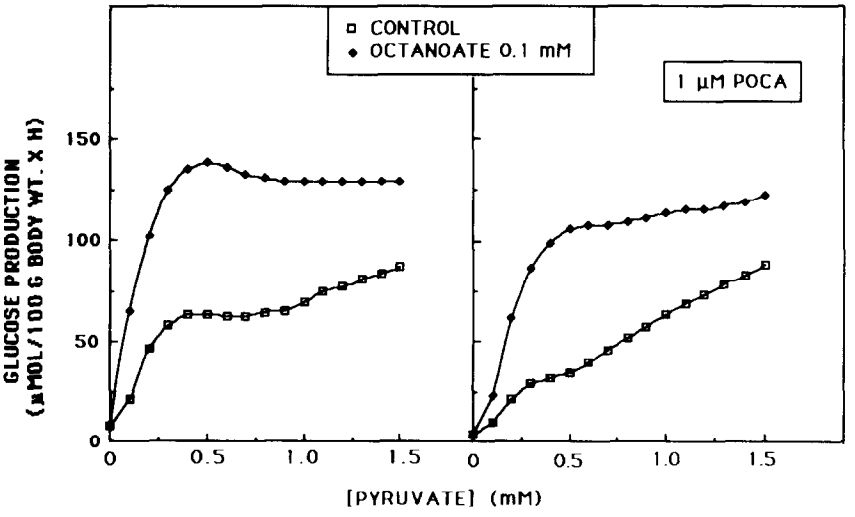

Fig. 4. Effect of octanoate on gluconeogenesis from increasing pyruvate concentrations in control and 2-5-4 chlorophenylpentyloxirane-2-carboxylate (POCA) treated livers. Livers from 48 hr starved rats were perfused with increasing concentrations of pyruvate (0–1.7 mM) in the presence (□) as well as in the absence (◆) of 0.1 mM octanoate as described in Figs 1 and 3. The left panel shows the effect of 0.1 mM octanoate in stimulating gluconeogenesis in control livers. In the right panel the effect of octanoate was tested in livers treated with 1 μM POCA. For the sake of clarity intermediate points have been omitted.

increasing pyruvate supply should lead to increasing oxaloacetate formation. The anaplerotic effect of increasing pyruvate carboxylation could counteract the energy deficiency brought about by POCA by increasing flux through the tricarboxylic acid cycle. It has been proposed that it is tricarboxylic acid cycle derived energy which is utilized to support gluconeogenesis [9, 20]. Therefore, it can be concluded that in the presence of POCA, if pyruvate availability is low, and thus its mitochondrial entry presumably restricted [21, 22], the intramitochondrial partitioning is shifted to its decarboxylation. Decreased rates of gluconeogenesis in this situation might be the result of a diminished intramitochondrial availability of pyruvate and/or decreased flux through the tricarboxylic acid cycle and thus useful energy for oxaloacetate formation. According to this view, POCA would inhibit gluconeogenesis by perturbing the mitochondrial partitioning of pyruvate between the carboxylating and decarboxylating reactions, as a result of changes in the mitochondrial NAD redox and/or phosphorylation potential. The physiological significance of our observations is determined by the fact that the pyruvate concentrations at which POCA inhibits gluconeogenesis are those found *in vivo*. Since the effectiveness of POCA in inhibiting gluconeogenesis is related to pyruvate availability, it seems plausible to conclude that the nutritional status and/or physical stress may significantly alter the therapeutic response to this drug.

Acknowledgements—The skilful technical assistance of Ms Ana Rodriguez and Marie José Arias-Salgado is greatly appreciated. We also appreciate the work of Mr Rafael Esteso in designing and building an oxygenator for the liver perfusion apparatus. This work has been supported in part by grants (87065 and PB87-0280) from Dirección General de Investigación Cientifica y Técnica, and Fondo de Investigaciones Sanitarias (88/1443 and 88/1450). C. González-Manchón was recipient of a fellowship from Ministerio de Educación y Ciencia.

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