Characterization of overt carnitine palmitoyltransferase in rat platelets; involvement of insulin on its regulation

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Received 30 May 1990; accepted 15 October 1990

Key words: diabetes, malonyl-CoA inhibition, rat platelets, insulin, carnitine palmitoyltransferase, saponin

Summary

Saponin-permeabilization (30/µg/ml) of the platelet plasma membrane, which enables access of added compounds to mitochondrial overt carnitine palmitoyltransferase (CPT I), was applied to allow the rapid determination of CPT I activity in situ. The effects of diabetes and short-term incubation with insulin in vitro on the kinetic parameters and malonyl-CoA sensitivity of CPT I were also studied in rat platelets. CPT I exhibited ordinary Michaelis-Menten kinetics when platelets were incubated with palmitoyl-CoA. Malonyl-CoA showed an I₅₀ (concentration giving 50% inhibition of CPT activity) of 0.92 ± 0.11/µM in permeabilized platelets. Platelets obtained from diabetic rats (induced by streptozotocin injection) exhibited an increased V₅₀ and I₅₀ for malonyl-CoA, and an unaltered Kₘ for palmitoyl-CoA. In contrast, preincubation of platelets prepared from both fed control rats and diabetic rats with insulin (100 and 150/µU/ml) led to a decrease in enzyme activity when assayed with 75/µM palmitoyl-CoA and 0.5 mM L-carnitine as substrates. These in vivo and in vitro results suggested that insulin directly modulated rat platelet CPT I activity, as it does in the liver.

Introduction

Platelets, like many other mammalian cells, take up long-chain fatty acids and metabolize them either to CO₂ via mitochondrial β-oxidation or to platelet lipid esters by esterification [1–4]. It is known that platelets contain various enzymes that metabolize fatty acids, including long-chain acyl-CoA synthetase (EC 6.2.1.3) [5], acetyl-CoA carboxylase (EC 6.4.1.2) [6], carnitine acetyltransferase (EC 2.3.1.7) [7], and carnitine palmitoyltransferase (CPT total, overt and latent; EC 2.3.1.21) [8].

It is generally agreed that hepatic CPT I, which is the overt form resides on the inner side of the mitochondrial outer membrane [9], appears to function as an important role in the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation [10]. The changes in CPT I activity is often accompanied by variations in enzyme sensitivity to malonyl-CoA which is the first intermediate of lipogenesis. Malonyl-CoA is known to a potent and probably a physiological inhibitor of CPT I [11]. Thus, it seems probable that the interrelationship between malonyl-CoA and CPT I is a major factor in regulating hepatic fatty acid oxidation in vivo.

However, there has been no previous report concerning the properties of CPT I and its sensitivity to malonyl-CoA inhibition in platelets. Hence the first aim of the present study is to develop a rapid method of in situ measurement of cellular CPT I
activity in the intact mitochondria of rat platelets permeabilized by saponin to understand both the kinetic parameters and sensitivity of CPT I to malonyl-CoA inhibition.

It has been shown that hepatic CPT I activity is modulated by some hormones such as insulin [12-15], glucagon [12, 15], and thyroid hormone [16]. A recent study by Guzmán & Geelen [12] showed that insulin lowered the CPT I activity and fatty acid oxidation in isolated rat hepatocytes after 30 min incubation period. Although it has been shown that platelet fatty acid oxidation is stimulated by thrombin [3] or by supplementation with L-carnitine [4], little is known about the hormonal regulation of fatty acid oxidation in resting and activated platelets. Because the platelet plasma membrane has high-affinity receptors for insulin [17], it can be anticipated that platelet CPT I also might be regulated by plasma insulin levels similar to the case of liver.

Thus changes on the kinetic parameters of CPT I in platelets were also investigated by studying the platelets obtained from streptozotocin-induced diabetic rats or by short-term incubation of platelets obtained from normal rats and diabetic rats with insulin in vitro to determine whether insulin might be involved in the regulation of CPT I in platelets.

**Materials and methods**

**Animals and induction of diabetes**

Male Wistar rats weighing 200–250 g were used in all experiments. Animals were kept in wire-bottomed cages under controlled temperature (22°C) conditions, with a 12 h dark/12 h light cycle. They were fed on a standard laboratory chow with food and water ad libitum before blood sampling. In some experiments, diabetes was induced by an intraperitoneal injection of streptozotocin (60 mg/kg) dissolved in 0.05 M sodium citrate buffer (pH 4.5). Control animals were injected intraperitoneally with the same buffer only. Streptozotocin-injected animals were allowed free access to food and water for the 48 hours following injection, after which blood samples were taken. Induction of diabetes was ascertained by measurement of the glucose in blood samples. Only animals having a blood glucose concentration higher than 15 mM were used.

**Preparation of platelets**

Platelets were prepared from whole blood. Blood samples, anticoagulated with 0.1 vol. of 3.8% (w/v) trisodium citrate, were collected from the inferior vena cava of rats anesthetized with pentobarbital sodium. The freshly collected blood was added to siliconized tubes and centrifuged at 130 g for 7 min at room temperature to obtain platelet-rich plasma (PRP). PRP was washed twice by centrifugation at 800 g for 15 min at room temperature with a solution containing 36 mM citric acid, 5 mM glucose, 5 mM KCl, and 90 mM NaCl, which was adjusted to pH 6.5 with NaOH. Prostaglandin E1 (PGE1) was added to this solution at a final concentration of 1 μM in order to prevent aggregation. The final platelet pellet was resuspended at a concentration of 5 × 10⁸ cells/ml in a modified Tyrode’s solution consisting of 11.9 mM NaHCO₃, 0.555 mM glucose, 2.68 mM KCl, 137 mM NaCl, 0.416 mM NaH₂PO₄, 1 mM MgCl₂, and 5 mM Hepes. The solution was adjusted to pH 7.35 with NaOH; and PGE₁ was added to a final concentration of 1 μM.

**Permeabilization of platelets**

The degree of permeabilization of the platelets was assessed by monitoring the leakage of marker enzymes from the cytosol (lactate dehydrogenase; LDH, EC 1.1.1.27) and mitochondria (glutamate dehydrogenase; GLDH, EC 1.4.1.3). Twice-washed platelets suspended in modified Tyrode’s solution were challenged with varying concentrations of saponin (0–100 μg/ml) at 37°C. Immediately after incubation for 0–10 min, the cells were sedimented by centrifugation at 2,400 g for 15 min at 4°C and the enzyme activity released into the supernatant was measured by a standard UV method (UV 2100, Shimadzu, Kyoto, Japan). For the de-