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SHORT COMMUNICATIONS

The Activity of β-Hydroxyacyl-CoA Dehydrogenase in the Pancreatic Islets of Hyperglycaemic Mice*

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Summary. The activity of β-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35.) was measured according to the principles of the Lowry microtechniques in pancreatic islets and acini, liver, heart and skeletal muscle in mice of the New Zealand obese strain (NZO) and the obese hyperglycaemic strain (obob). The assayed enzyme showed the same pH dependence in material from islets, liver and heart muscle. The highest enzymatic activity was found in pancreatic islets and heart muscle, and this was 2 to 5 times the activity obtained in pancreatic acini, liver and skeletal muscle. The results show that one prerequisite for a fast β-oxidation of fatty acids exists in the islets of Langerhans.

L'activité de la β-hydroxyacyl-CoA déshydrogénase dans les îlots pancréatiques de souris hyperglycémiques

Résumé. L'activité de la β-hydroxyacyl-CoA déshydrogénase (EC 1.1.1.35.) a été mesurée, selon les principes de microtechniques de Lowry, dans les îlots et les acini pancréatiques, le foie, le muscle cardiaque et le muscle du squelette chez des souris de la souche obèse de Nouvelle-Zélande (NZO) et de la souche obèse hyperglycémique (obob). L'enzyme dosé montrait la même dépendance du pH dans les îlots, le foie et le muscle cardiaque. L'activité enzymatique la plus élevée a été trouvée dans les îlots pancréatiques et le muscle cardiaque, et représentait 2 à 5 fois l'activité obtenue dans les acini pancréatiques, le foie et le muscle du squelette. Les résultats montrent qu'une condition nécessaire pour une β-oxydation rapide des acides gras existe dans les îlots de Langerhans.

Die Aktivität der β-Hydroxyacyl-CoA Dehydrogenase in Pankreasinseln von hyperglykämischen Mäusen

Zusammenfassung. Die Aktivität der β-Hydroxyacyl-CoA Dehydrogenase (EC 1.1.1.35.) wurde nach den Prinzipien der Lowry-Mikrotechniken in den Pankreasinseln und -Acini, der Leber, dem Herzen und dem Skeletmuskel von Mäusen des fettsüchtigen New Zealand-Stammes (NZO) und in dem fettsüchtig-hyperglykämischen Stamm (obob) gemessen. Das untersuchte Enzym zeigte die gleiche pH-Abhängigkeit in Material aus den Inseln der Leber und dem Herzmuskel. Die höchste Enzymaktivität fand sich in Pankreasinseln und Herzmuskel; sie war zwei bis fünfmal höher als in den Pankreas-Azini, der Leber und der Skeletmuskulatur. Die Ergebnisse zeigen, daß eine Voraussetzung für eine schnelle β-Oxydation von Fettsäuren in den Langerhans'schen Inseln erfüllt ist.

Key-words; Hyperglycaemic syndrome of mice (NZO, obob), β-oxidation of fatty acids, β-hydroxyacyl-CoA dehydrogenase, islets of Langerhans, liver, heart and skeletal muscle.

The blood glucose is tightly regulated by the B-cells in the islets of Langerhans, and even a small variation in the concentration of blood glucose influences the release of insulin from the islets [4]. Another mechanism affecting the glucose homeostasis is the glucose-fatty acid cycle of Randle *et al.* [10] which does not involve the participation of the B-cells. Furthermore, Malaisse and Malaisse-Lagae [7] have recently found a direct effect of palmitate on the insulin release in rat tissue *in vitro*, and others [11, 8] have shown the same for octanoate. The insulin release may be initiated by increased concentrations of fatty acids or some of their metabolites in the B-cells. The β-oxidation of fatty acids is of importance as the primary step in the degradation of fatty acids. The enzymatic prerequisites for this metabolic pathway have not been studied in the islets. One of the enzymes in this cycle, β-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35.), has therefore been chosen as a first step in the evaluation of the degradation of fatty acids in the islets. In comparison, pancreatic acini, liver, heart and skeletal muscle have been included in the study of the islets in mice.

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Material and Methods

Four female mice of the New Zealand obese strain, NZO [2], 6 months old, weighing 41—53 g have been used together with three female mice, 6 months old, with the obese hyperglycaemic syndrome (obob) and two lean litter mates (Obob, ObOb) from a strain originating at the R.B. Jackson Memorial Laboratory, Bar Harbor, Maine, U.S.A. Their weights were 53—69 g and 24—27 g respectively. The material comprised pancreatic acini and islets (cauda), liver (right lobe), heart muscle (left ventricle) and skeletal muscle (quadriceps). The handling of the material was performed according to the principles of Lowry [5] with dissection of freeze-dried sections obtained from the various tissues.

Incubation conditions. The assay of β-hydroxyacyl-CoA dehydrogenase was a modified form of the method described by Adachi *et al.* [1]. By this method the oxidation of a β-hydroxyacyl-CoA derivative to the

corresponding ketoacyl-CoA derivative in the mitochondrion could be reversed if the pH was decreased below 7. The product measured in this system was the oxidized nicotineamide-adenine dinucleotide. The reduction of acetoacetyl-CoA could consequently be used to measure the activity of β-hydroxyacyl-CoA dehydrogenase. The final medium consisted of 0.44 mM acetoacetyl-CoA,[1] 1.0 mM nicotineamide-adenine dinucleotide[2] and 0.05% bovine plasma albumin[3] suspended in 0.06 M pyrophosphate buffer, pH 6.0 with a total volume of 8.08 µl. In a study of the pH dependence of the enzymatic reaction, pyrophosphate buffer was used in the pH-range 6.0—8.1 and 0.1 M citrate buffer was used in the pH-range 3.9—6.0. The time of this incubation was 10 min. The fluorimetric measurements of the oxidized dinucleotide were made in a Farrand A fluorimeter according to Lowry *et al.* [6]. The enzymatic activity was expressed as moles of substrate converted per kg dry weight and hour of incubation (MKH).

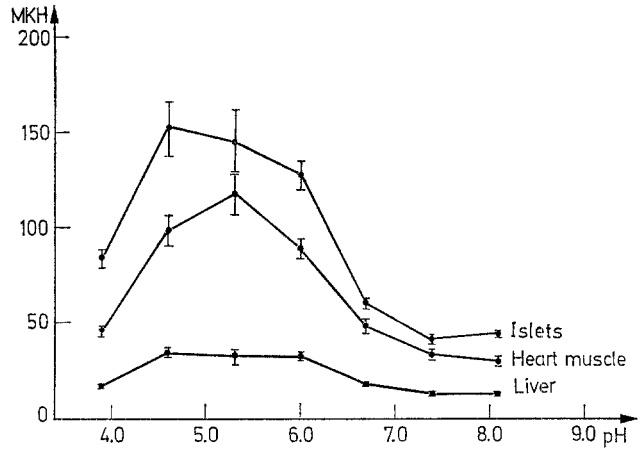

Fig. 1. The activity of β-hydroxyacyl-CoA dehydrogenase in heart muscle, liver and islets of Langerhans of a NZO-mouse as related to pH. Means and standard errors obtained from five determinations are given

Results

The pH dependence of the enzymatic reaction and the consumption of the substrate were measured in tissues from NZO-mice. The pH of the incubation media was varied between 3.9 and 8.1. The optimal activity was obtained in the pH interval between 4.6 and 6.0 for heart muscle, liver and islets, as shown in Fig. 1. The consumption of the substrate showed a linear dependence on the sample weights between 40 and 200 ng for islets, liver, pancreatic acini and skeletal muscle, as shown in Fig. 2. The error of the determinations varied between 12 and 15 per cent in the islets and the acini, and it was 5 per cent in the liver and the heart muscle.

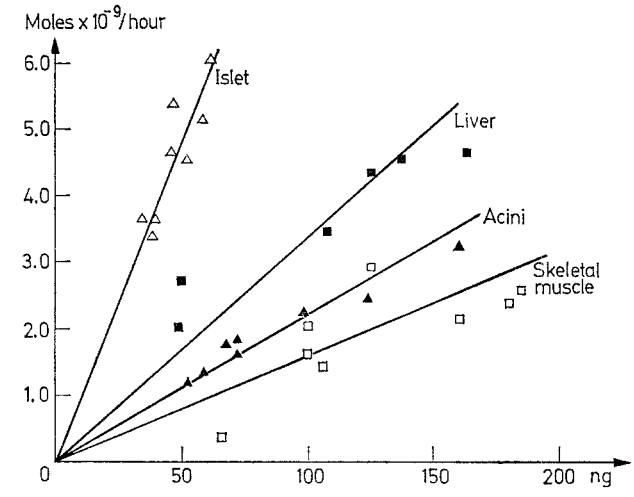

Fig. 2. Consumption of the substrate in pancreatic islets and acini, liver and skeletal muscle of a NZO-mouse as related to the amount of tissue incubated

The results of the assays are summarized in Table 1. In the NZO-mice the highest activity was obtained in the pancreatic islets and lower activities were found in heart and skeletal muscle, liver and pancreatic acini. In the obese hyperglycaemic mice (obob) the heart muscle displayed a higher enzymatic activity than the islets did. The highest values for the enzymatic activity found was seen in pancreatic islets of the two lean litter-mates (Obob, ObOb).

Table 1. *Enzymatic activities of β-hydroxyacyl-CoA dehydrogenase expressed as moles of subtrate converted per kg dry weight and hour of incubation (MKH)*
Mean values and ranges

	Pancreatic islets	Pancreatic acini	Liver	Heart muscle	Skeletal muscle
New Zealand Obese (NZO) (4)[a]	122 (105[b]—133)	21 (19—26)	34 (32—35)	85 (72—97)	22 (7—38)
Obese hyperglycaemic (obob) (3)	99 (87—113)	18 (14—24)	46[c] (45—48)	112 (91—140)	16[c] (9—22)
Lean litter-mates (Obob, ObOb (2)	160 (139—181)	19 (14—25)	47 (43—51)	102 (86—117)	6[c]

[a] Number of animals [b] Mean of 5 observations in each animal [c] One value lost

1 Sigma Chemical Co, St. Louis, Missouri, USA.
2 C.F. Boehringer und Soehne, Mannheim, Germany.
3 Armour Pharmaceutical Co, Eastbourne, England.

Discussion

The control experiments, shown in Fig. 1, display a similar pH dependence of β-hydroxyacyl-CoA dehydrogenase for the different tissues investigated. In the presented material, the activity varied with pH in a way similar to that observed in the skin by Adachi *et al.* [1].

The experimental errors obtained for the different tissues were acceptable except for that of the skeletal muscle (Table 1). One reason for the great variation obtained in skeletal muscle is the uneven distribution of the mitochondria within the fibres, which was not allowed for during the dissections.

The results given in Table 1 showed that a considerable activity of the β-hydroxyacyl-CoA dehydrogenase was present in the islets of Langerhans. The activity was also high in heart muscle, which has a strong demand for β-oxidation of fatty acids during starvation [9, 3]. The results suggest therefore, that a fast β-oxidation of fatty acids exists in the islets. This may be of importance for regulation of the insulin release.

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