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The Influence of Tolbutamide on Fatty Acid Metabolism in the Liver and Muscle

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Summary

Metabolism of palmitate-^{14}C was studied in the rat liver and muscle incubated with 1 mmol.l^{-1} tolbutamide *in vitro* experiments: Tolbutamide reduces the utilization of free fatty acids in the liver by inhibiting their uptake, incorporation into total lipids, and oxidation to $^{14}CO_2$. Tolbutamide stimulates the incorporation into the triacylglycerol fraction in individual liver lipid fractions and inhibits the incorporation into the free fatty acid fraction. As in the liver, tolbutamide inhibits the uptake, incorporation into total lipids, and oxidation to $^{14}CO_2$ in the muscle. In individual lipid fractions, tolbutamide only inhibits the incorporation of palmitate into cholesterol esters. It can be concluded that tolbutamide directly interferes with fatty acid metabolism and thus improves glucose utilization and insulin resistance.

Key words

Rats – Liver – Muscle – Tolbutamide – Na-palmitate-1^{14}C metabolism

Introduction

Recent findings on the pathogenesis of atherosclerosis indicate that insulin resistance and consequent hyperinsulinaemia are primary importance in the development of atherosclerosis (Reaven 1991). It is the aim of atherosclerosis prevention to avoid hyperinsulinaemia by limiting insulin administration. This had increased the significance of sulfonylureic antidiabetics.

After the sulfonylureic antidiabetics were introduced mainly because of their stimulatory effect on insulin secretion (Blackward and Nelson 1971). At present, we stress their direct effect on glucose utilization in skeletal muscles. While the influence of sulfonylureic antidiabetics on glucose metabolism is unambiguous, their effects on lipid metabolism, namely fatty acids, is less evident and the results are controversial. Experimental studies (Cook 1987, McCormick *et al.* 1986, Patel 1986) have demonstrated that the oxidation of fatty acids in the liver, muscles, and the heart of normal and diabetic rats is inhibited. Nevertheless, the experiments of Kawazu *et al.* (1980) were contrary to this inhibition.

In this study, we investigated the influence of tolbutamide on fatty acid metabolism in the liver and diaphragm during experiments *in vitro* with Na-palmitate-1^{14}C. Results of the study show that tolbutamide reduces fatty acid utilization in the liver and muscle by the inhibition of uptake, incorporation of palmitate-1^{14}C into lipids, and oxidation to $^{14}CO_2$.

Material and Methods

Animals

The tissues were obtained from male rats of the Wistar strain (Dobrá Voda) weighing 160–180 g, which were fed a standard laboratory diet *ad libitum*. They were housed in an air-conditioned room maintained at 21±2 °C under a 12/12 h light-dark cycle. Animals were exsanguined after 20 h of starvation, liver slices were prepared by free hand, and the diaphragm was initially incubated after separation into two parts.

Procedure of incubation for free fatty acid uptake

Liver slices and the hemidiaphragm were incubated in Ringer-bicarbonate solution at pH 7.4, containing 1.0 mmol.l^{-1} Na-palmitate (2.86 MBq), 1 % bovine albumin (fraction V), 5 mmol.l^{-1} glucose, and 1 mmol.l^{-1} tolbutamide after washing in cold physiological saline. The control solution had the same

composition but without tolbutamide. Na-palmitate-$1^{14}C$ was prepared by the procedure according to Milstein and Driscoll (1959). Incubation was carried out under optimal conditions for 60 min at 37 °C in 95 % O_2 and 5 % CO_2 (Chorvathová *et al.* 1976). After incubation, released $^{14}CO_2$ was absorbed into an ethanolamine solution and determined according to Saba Di Luzio in the modification of Nemec *et al.* (1971). Tissue activity illustrating the incorporation of Na-palmitate-$1^{14}C$ was measured in an aliquot of lipid extract obtained from standard decontaminated tissue by the method of Folch *et al.* (1957). The individual lipid fractions were separated by thin-layer chromatography on Kieselgel H (Type 60, Merck) by means of the separating system ether : petrolether : acetic acid (20:79:1) (Boberg 1966). We pooled the activities of all the separated fractions: free fatty acids (FFA), mono- and diglycerides (MG + DG), triacylglycerols (TG), cholesterol esters (CHE), and phospholipids (PL). They were measured by liquid scintillation spectrophotometer (Nuclear Chicago) and expressed in dpm per gramme of tissue.

Further details of the metodical procedure are given elsewhere (Chorváthová *et al.* 1976). The results of the palmitate uptake (the sum of incorporation into total lipids and oxidation to CO_2) are expressed as the percentage dpm value per gramme of tissue in relation to the value in the incubation medium. The results represent values from 9–11 animals and were statistically evaluated by Student's t-test.

Table 1
Utilization of palmitate-$1^{14}C$ in the liver and diaphragm (% dpm/g)

	Uptake	Total lipids	CO_2
Liver			
Control (11)	16.26 ± 0.65	15.91 ± 0.65	0.35 ± 0.02
Tolbutamide (11)	12.67 ± 0.76[a]	12.46 ± 0.75[a]	0.21 ± 0.01[a]
Hemidiaphragm			
Control (11)	6.05 ± 0.26	5.22 ± 0.26	0.83 ± 0.03
Tolbutamide (11)	4.31 ± 0.18[b]	3.65 ± 0.17[b]	0.65 ± 0.02[b]

Statistical significance: [a]$p < 0.01$, [b]$p < 0.001$

Table 2
Incorporation of palmitate-1-^{14C} into the liver and diaphragm lipid fractions (dpm/g)

	Liver		Hemidiaphragm	
	Control (9)	Tolbutamide (9)	Control (9)	Tolbutamide (9)
PL	53.40 ± 8.17	43.10 ± 5.92	20.76 ± 8.66	24.00 ± 2.21
MG + DG	38.10 ± 3.01	38.03 ± 4.63	22.29 ± 2.17	21.61 ± 1.28
TG	34.56 ± 3.52	48.40 ± 3.89[a]	23.67 ± 2.32	23.66 ± 2.47
FFA	470.16 ± 53.76	341.48 ± 20.71[a]	95.71 ± 9.43	85.27 ± 9.55
CHE	46.87 ± 2.93	40.36 ± 6.58	46.01 ± 3.69	31.01 ± 3.21[b]

Statistical significance: [a]$p < 0.05$, [b]$p < 0.01$

Results

Tolbutamide had the following effects on the uptake and utilization of Na-palmitate-$1^{14}C$.

Liver. We observed in *in vitro* experiments that the uptake of Na-palmitate by liver slices is lower in the presence of 1 $mmol.l^{-1}$ tolbutamide, as well as its incorporation into total lipids and its oxidation to $^{14}CO_2$ (Table 1). In individual lipid fractions, tolbutamide stimulated incorporation into the triacylglycerol fraction and inhibited incorporation into the free fatty acids fraction. The decreased

incorporation of palmitate into total lipids concerned both a significant decrease of the activity of the free fatty acid fraction and the statistically non-significant decrease of activity of phospholipids and cholesterol esters (Table 2).

Diaphragm. Tolbutamide inhibited Na-palmitate uptake, the incorporation into lipids and oxidation to $^{14}CO_2$ in the diaphragm (Table 1). It also inhibited incorporation of Na-palmitate into the cholesterol esters from individual lipid fractions. A significant reduction of activity of cholesterol ester fraction and a non-significant decrease of free fatty acids participated in the decreased incorporation of palmitate into total lipids (Table 2).

Discussion

The above results show that tolbutamide can influence not only glucose metabolism, but that it also affects the metabolism of lipids, especially fatty acids directly. Its effect is considerable in both liver and muscle where fatty acid utilization is reduced by inhibition of uptake, incorporation into lipids, and oxidation to $^{14}CO_2$.

Liver. Our results agree with the perfussion and mitochondrial studies which reported reduced formation of ketone bodies as the main products of fatty acid oxidation in the liver (Patel 1986a, Mc Cormick *et al.* 1986). Inhibition of fatty acids oxidation is not dependent on the inhibition of lipolysis as was assumed previously (Hasselblatt 1969), but directly on the inhibition of carnitine palmitoyltransferase activity in competition with L-carnitine (Patel 1986, Cook 1987). The decrease of fatty acid oxidation in the liver is important, because this drug inhibits ketogenesis (Patel 1986b) at the same time with the consequent reduction of ketone bodies in the blood, as well as hepatic gluconeogenesis, which is stimulated by fatty acids oxidation (Patel *et al.* 1984).

Skeletal muscle. The inhibitory effect of tolbutamide on fatty acid oxidation is also documented by reduced CO_2 production in studies with perfused myocardium (Kramer *et al.* 1983) and skeletal muscle (Daniels and Lewis 1982). Reduced fatty acid oxidation can activate Randle's cycle (Randle *et al.* 1963) to higher glucose oxidation. Improved glucose utilization and lowered glycaemia are stimuli for reducing insulin levels. By this mechanism, sulfonylureic antidiabetics helps to improve insulin resistance (Tan *et al.* 1984).

Physiological relevance. Insulin resistance is one of the most important risk and pathogenic factors in atherosclerosis (Reaven 1988). Research on insulin sensitizers is oriented towards the direct influence of glucose metabolism (Faber-ole *et al.* 1990) on the one hand and the inhibition of fatty acid oxidation on the other (Foley 1992). Tolbutamide and other sulfonylureic antidiabetics are unique since they influence both metabolic pathways directly. The first clinical observations have shown that its effect can also be demonstrated in patients (Dzúrik *et al.* 1991).

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