CONTROL OF PYRUVATE CARBOXYLASE IN ALLOXAN DIABETIC RATS

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SUMMARY: In order to contribute to a clarification of the contradictory reports from different laboratories concerning the control of pyruvate carboxylase in diabetes, the levels of this enzyme were determined in rat liver at different stadiums of alloxan diabetes. In agreement with the former reports unchanged and elevated levels of pyruvate carboxylase were found at the early and late stadium of diabetes, respectively. The implications of this finding concerning a possible role of glucocorticoids in the control of gluconeogenesis in diabetes, is discussed.

Increased incorporation of lactate (1), pyruvate and carbon dioxide (2 - 5), into glucose in experimental diabetes is associated with an elevation of the four key enzymes of gluconeogenesis from pyruvate or lactate, namely pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase (for a summary see 6 - 8). Contrary to numerous reports, an adaptive response of pyruvate carboxylase has been questioned by several authors based upon unaltered levels of this enzyme found in livers of the diabetic rat (9 - 12). This finding has been considered as an additional support for an exclusive location of pyruvate carboxylase in mitochondria (11).

Studies on the intracellular location of liver pyruvate carboxylase in our laboratory resulted in isolation of a subcellular particle which lacks an enzyme considered so far as mitochondrial, but still contains pyruvate carboxylase (13). In a systematic study of the relative content of this subcellular population in liver at different metabolic states it turned out, that pyruvate carboxylase increases only at a later stadium of the diabetic state. In the hope to clearify the apparent discrepancies between our results and those reported from LARDY'S and KREBS' laboratories concerning the control of pyruvate carboxylase in the diabetic state the levels of this enzyme were investigated in the acute and chronical stadium of alloxan diabetes (14). As expected, these studies revealed unchanged levels at the acute diabetic stadium but elevated levels at the chronical stadium.

MATERIAL AND METHODS

Male albino rats of the Sprague Dawley strain fed on rat pellets (Atromin GmbH, Lage/Lippe) and weighing 180 - 220 g were used for these studies. Diabetes was produced by injection of 65 mg alloxan tetrahydrate (Merck, Darmstadt) per kg body weight in 0.9% sodium chloride under light ether anesthesia into the femoral vein of rats starved for 24 hours.

For a study of pyruvate carboxylase in the acute diabetic stadium, blood glucose levels were determined two days later. All animals showing blood sugar levels above 400 mg/per 100 ml were used.

A second group of animals in the acute diabetic stadium received two units depot insulin (Hormon-Chemie, München) twice a day per animal for one week. This dose was continuously reduced during the second week. After two additional days without insulin the animals were used for the study of the liver pyruvate carboxylase (chronical stadium of diabetes).

The animals were killed in light ether anesthesia; the livers were removed and homogenized in 1o volumes of o,25 M sucrose containing o.1% sodium desoxycholate. After standing for one hour at $0^{o}C$ to solubilize pyruvate carboxylase completely the enzyme activity was determined according to HENNING and SEUBERT (15).

Blood glucose was determined in both groups after 12 hours starvation. Control animals received instead of alloxan o.9% sodium chloride solution.

RESULTS AND DISCUSSION

In the table the levels of pyruvate carboxylase activities in liver and blood glucose at the various metabolic states of diabetes are summarized.

As is evident, in the acute diabetic stadium the levels of pyruvate carboxylase are not elevated ($p>o.o5$) although blood glucose is greatly increased ($p<o.oo1$). Only after a longer period elevation of pyruvate carboxylase can bei observed ($p<o.oo1$). Insulin gifts as specified in the experimental part during this period turned out to be

T A B L E

Pyruvate carboxylase and blood glucose levels in normal and alloxan diabetic rats, 48 hours and 16 days after injection of alloxan.

Experimental group	Blood glucose in mg/1oo ml	Pyruvate carboxylase U./liver wwt.	Pyruvate carboxylase U./mg protein
Controls (6)	15o 149 135 124 135 15o	3.75 4.62 4.o2 5.36 4.42 4.42	o.o29 o.o31 o.o24 o.o3o o.o23 o.o23
	$\bar{x}$ = 14o.4 S.E.M.=±4.5	$\bar{x}$ = 4.43 S.E.M.=±o.22	$\bar{x}$ = o.o266 S.E.M.=±o.oo15
Acute diabetic stadium (7)	514 466 956 454 96o 483 461	5.25 5.47 4.75 4.51 4.58 6.7o 5.15	o.o28 o.o28 o.o33 o.o34 o.o25 o.o38 o.o3o
	$\bar{x}$ = 613.4 S.E.M.=±89.3	$\bar{x}$ = 5.2o S.E.M.=±o.28	$\bar{x}$ = o.o3o1 S.E.M.=±o.oo17
Chronical diabetic stadium (8)	437 49o 88o 522 534 424 911 411	9.16 9.16 8.96 8.82 11.83 9.6o 8.o4 8.93	o.o48 o.o47 o.o51 o.o49 o.o65 o.o54 o.o45 o.o5o
	$\bar{x}$ = 576.1 S.E.M.=±71.5	$\bar{x}$ = 9.31 S.E.M.=±o.39	$\bar{x}$ = o.o51 S.E.M.=±o.oo22

Number of experiments is given in parenthesis.

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necessary, because most animals would have died due to the acute ketosis.

In both groups with diabetes blood glucose was assayed after a period of 12 hours starvation. Elevated glucose levels under these conditions in the acute diabetic stadium, followed only after a lag period by an increase of pyruvate carboxylase levels, therefore point to different mechanism participating in the control of gluconeogenesis in diabetes. Similar conclusions have been drawn from studies on the control of gluconeogenesis by cylo-AMP and glucocorticoids. Apparently a short time effect most likely due to an increase of cylo-AMP (16) resulting in acceleration of gluconeogenesis (17) is followed by a long term effect associated with induction of glucogenic enzymes. Participation of the latter effect in control of gluconeogenesis is supported by the delayed normalization of both gluconeogenesis from pyruvate (2) and the levels of glucogenic enzymes (2, 18, 19) after insulin injections. Control of the inactivation of glucocorticoids by insulin resulting in elevated levels of these hormones in liver might be the primary cause of insulin deficiency on the activities of pyruvate carboxylase and the other glucogenic enzymes (2o).

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