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EFFECT OF EXPERIMENTAL DIABETES ON ORNITHINE DECARBOXYLASE ACTIVITY OF RAT TISSUES

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SUMMARY: Measurements have been made of the activity of ornithine decarboxylase of liver, heart, kidney and brain in alloxan-diabetic and control rats. In all these tissues this enzyme had decreased markedly at four weeks after induction of diabetes. These results are discussed in relation to the hormonal control and cyclic nucleotide regulation of ornithine decarboxylase.

A wide range of hormones have been implicated in the control of ornithine decarboxylase (L-ornithine carboxy-lyase; EC 4.1.1.17)(ODC) [1-6]. It has been proposed that hormones and other agents raising ODC activity might have a common mediator in cAMP [7-9]. ODC has a short half life [10] and most studies have been concerned with rapid, short term effects of hormones on this key enzyme of polyamine synthesis. It has been shown that hepatic ODC is increased by insulin, glucagon, growth hormone, hydrocortisone and thyroxine [1]; synergistic effects of two hormones have been noted in a number of cases [2,4,11]. Parallel increases in ODC activity have been observed in a number of tissues in response to a particular hormone e.g. growth hormone stimulation of ODC in heart, kidney, liver, thymus, lung, spleen and adipose tissue [12].

Abbreviations: ODC, ornithine decarboxylase (L-ornithine carboxy-lyase)(EC 4.1.1.17); cAMP, adenosine 3':5'-monophosphate; cGMP, guanosine 3':5'-monophosphate.

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Diabetes is a condition of some interest in relation to ODC activity in that the hormonal profile is altered for a prolonged period of time with substantial changes in growth and protein synthesis. In diabetes, in addition to the decrease in insulin, there is evidence for an elevated plasma growth hormone [13] and glucagon [14], all three of which are known to be involved in the regulation of ODC [6].

In the present study, measurements were made of the activity of ODC in liver, heart, kidney and brain in alloxan-diabetic and control rats. These tissues were selected as representing a spectrum of response to diabetes; liver and heart are considered to be insulin sensitive tissues, while brain and kidney are relatively independent of the direct action of insulin.

METHODS

Adult male albino rats of the Wistar strain were used, the initial body weight was 220-250g. The rats were starved for 48hr and alloxan monohydrate, dissolved in 0.1M sodium acetate buffer pH 4.5, was injected subcutaneously, the dose was 20mg/100g body weight. Thereafter, 2 units of protamine zinc insulin were injected daily for 1 week. Insulin was then withdrawn and the rats used 2-3 weeks later. Streptozotocin was injected intravenously at a dose of 6.5mg/100g body weight.

Tissue homogenates were prepared with 4 volumes of ice-cold medium containing 0.25M sucrose, 20mM triethanolamine buffer pH 7.4, 5mM dithiothreitol and 0.2mM pyridoxal phosphate. A supernatant fraction was obtained after centrifugation for 15 min at 40,000g. ODC was measured by a modification of the methods of Pegg and Williams-Ashman [15] and Oka and Perry [4]. The reaction mixture contained, in a final volume of 1 ml, the following components: 100μmoles of glycylglycine buffer pH 7.2; 5μmoles dithiothreitol; 0.2μmoles pyridoxal phosphate; 0.25μCi L-[1-^{14}C]ornithine and 30nmoles cold ornithine. 0.2ml tissue extract was added and incubated for 30 min at 37°C. The reaction was stopped by addition of 0.2ml of 5N perchloric acid, the $^{14}CO_2$ was collected in the centre well in 0.5ml Hyamine. Blank determinations were made as above without the tissue extract. L-[1-^{14}C]ornithine was treated with acid and freeze-dried essentially as described by Oka and Perry [4] to reduce the blank value.

One unit of ornithine decarboxylase activity was defined as a nmol of $^{14}CO_2$ liberated from the carboxyl-labelled L-ornithine in 30 min at 37°C. L-[1-^{14}C]ornithine was obtained from the Radiochemical Centre, Amersham, Bucks.

Table 1. Effect of alloxan-diabetes on tissue weight and ornithine decarboxylase activity

	Control	Alloxan-diabetes	Diabetes as % of control	P
Blood sugar (mg%)	91 ± 10 (12)	570 ± 17 (12)		
Body weight (g)	354 ± 12 (27)	212 ± 9 (30)	59	***
Liver weight (g)	14.50 ± 0.45 (21)	9.91 ± 0.38 (22)	68	***
Kidney weight (g)	2.76 ± 0.13 (17)	3.56 ± 0.21 (19)	129	***
Heart weight (g)	1.03 ± 0.04 (13)	0.72 ± 0.04 (14)	70	***
Brain weight (g)	1.37 ± 0.03 (18)	1.19 ± 0.02 (19)	87	***
Adipose - 2 fat pads - weight (g)	3.71 ± 0.32 (14)	0.50 ± 0.09 (16)	13	***
Ornithine decarboxylase activity (U/g)				
Liver	0.180 ± 0.019 (18)	0.127 ± 0.019 (15)	70	*
Kidney	2.77 ± 0.256 (14)	0.588 ± 0.147 (16)	21	***
Heart	0.709 ± 0.164 (7)	0.163 ± 0.037 (8)	23	***
Brain	0.183 ± 0.013 (13)	0.086 ± 0.021 (11)	47	***
Ornithine decarboxylase activity (Total U/organ)				
Liver	2.58 ± 0.32 (18)	1.31 ± 0.21 (15)	51	**
Kidney	7.65 ± 0.71 (14)	2.09 ± 0.52 (16)	27	***
Heart	0.791 ± 0.291 (7)	0.122 ± 0.031 (8)	15	***
Brain	0.249 ± 0.051 (13)	0.100 ± 0.024 (11)	40	***

Values are given as means ± SEM. Fisher's P values are shown by asterisks; *$P<0.05$; **$P<0.01$; ***$P<0.001$. No. of observations are given in parentheses.

RESULTS AND DISCUSSION

ODC is strikingly decreased in all the tissues examined from alloxan diabetic rats (Table 1), these results suggest that insulin is an essential component of the spectrum of hormones regulating this enzyme. Alloxan-diabetic rats maintained on protamine zinc insulin (2 i.u/daily for 10 days) retained the normal tissue ODC activity.

ODC activity of kidney and liver of streptozotocin-treated rats were 65% and 47% of control values respectively (based on units/g tissue). The smaller response of kidney ODC may well be related to the less severe form of diabetes induced by this dose of streptozotocin.

The present results, despite the difference in time scale involved, are complementary to those of Panko and Kenney [1], who demonstrated the action of insulin in increasing hepatic

ODC of adrenalectomized rats, and those of Aisbitt and Barry [16], Hogan et al. [17], Oka and Perry [4], and Yamasaki and Ichihara [18] who have shown increases in ODC in various cell cultures after addition of insulin.

Three factors, known to be associated with stimulation of ODC activity, are increased in diabetes; these are, plasma glucagon and growth hormone [13,14] and cAMP in liver and adipose tissue [19,20]. The present finding that ODC is markedly decreased in a wide range of tissues from diabetic rats emphasises the overriding importance of insulin in the control of this enzyme.

In considering the spectrum of compounds that raise tissue ODC activity [6,18,21] it is tempting to speculate that insulin, growth hormone and amino acids might have a concerted action on the regulation of this step in polyamine synthesis. The observations (a) that administration of amino acids in vivo or to cell cultures or suspensions in vitro or the administration of insulin and amino acids, all increase ODC activity [17,18,22]; (b) that amino acids stimulate the secretion of insulin, growth hormone and glucagon [14,23,24]; (c) that insulin and growth hormone stimulate ODC activity in a number of tissues [1,4,12, 17,18,25]; (d) that insulin and growth hormone have synergistic effects on amino acid metabolism and protein synthesis (contrasting with their opposing actions in the disposition of glucose and fatty acids)[23,24,26]; (e) that insulin increases amino acid uptake by tissues [27,28]; all point to the close and integrated association of insulin, growth hormone and amino acids in the regulation of ODC. The present observations support this hypothesis and suggest that insulin is necessary for the expression of growth hormone action. It may be noted, in paren-

thesis, that arginine is not only the substrate for arginase and the immediate precursor of intracellular ornithine, but it also stimulates the secretion of insulin, growth hormone and glucagon. Thus a focal point in the regulation of ODC activity may centre upon arginine metabolism. It may also be argued that arginine acts as a signal in the regulation of ODC activity in those tissues where insulin is not thought to have a direct action, such as brain and kidney. The high Km of arginase [29] would make the production of ornithine very sensitive to changes in arginine concentration.

The present results may also provide some indirect evidence relating to the question of the role of cyclic nucleotides in the regulation of ODC. The elevated hepatic and adipose tissue cAMP [19,20] and diminished ODC of a range of tissues, including liver and adipose tissue, in diabetes do not readily fit the hypothesis that cAMP is the common mediator for the multiplicity of factors regulating ODC [30,31]. Eloranta and Raina [3] have summarised much of the evidence for cAMP as a common factor for hormonal control of ODC and they have concluded that it is unlikely that nucleotide plays this central role, although they do not exclude cGMP as the common factor.

The role of cGMP as a regulator of ODC activity is attractive in relation to the present changes observed in diabetes. The effect of insulin in raising cGMP in liver and adipose tissues [32], the lowering of hepatic cAMP as the blood insulin/glucagon ratio changes [33], the effect of insulin in increasing cAMP phosphodiesterase [34-36], all indicate a possible role for cGMP, or for changes in the cGMP/cAMP quotient, in mediating the effects of insulin. Oka and Perry [4] have suggested, from studies of the biphasic response of ODC during hormone-dependent

development of mammary gland epithelial cells, that regulation may be exerted by insulin and prolactin independent of cAMP.

The present work stresses the synergism between insulin and pituitary hormones in the control of ODC over a prolonged period of time. In the absence of insulin, or of the pituitary gland [2] ODC decreases in activity. This may be an important facet of the disturbance of regulatory processes in cell function which occurs in diabetes.

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