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EFFECT OF INSULIN ON THE METABOLISM OF L-ASCORBIC ACID IN ANIMALS

PRABIR K. MAJUMDER, SUDIP K. BANERJEE, RAMAN K. ROY and GORA C. CHATTERJEE
Department of Biochemistry, University College of Science, Calcutta-19, India

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Abstract—Several enzymes of L-ascorbic acid metabolism have been estimated in the tissues of rats under conditions of alloxan diabetes and subsequent treatment with either insulin, insulin and actinomycin D or with insulin and cycloheximide. The enzyme "gulonooxidase" which is responsible for the biosynthesis of L-ascorbic acid from L-gulonolactone was found to be regulated by insulin at the post-transcriptional level. No pronounced effect was noted in the case of various other enzymes like dehydroascorbatase, uronolactonase, gulonate dehydrogenase and gulonate decarboxylase in rats under the identical experimental conditions.

RECENTLY studies have been carried out in animals on the metabolism of L-ascorbic acid under varying nutritional conditions[1–3] particularly on the regulatory role of dietary proteins on these enzymes.[4–6] Insulin is known to act as a suppressor of key gluconeogenic enzymes and also as an inducer of glucokinase.[6–8] In the present communication investigations have been carried out on the effect of alloxan diabetes and subsequent administration of either insulin, insulin and actinomycin D, or of insulin and cycloheximide on the enzymes involved in the metabolism of L-ascorbic acid in rats.

MATERIALS AND METHODS

Chemicals. Actinomycin D was obtained as a gift from Merck Sharp Dohme, U.S.A. Actidione (cycloheximide) was purchased from the Upjohn Company, Michigan, U.S.A. Alloxan used was made by BDH, England and insulin (Protamine zinc) was purchased from Boots (India) Ltd.

Animal experiments. Male albino rats weighing 110–120 g were used in the experiment and were maintained on laboratory stock diet *ad lib.*[1] All the rats were fasted for 24 hr; diabetes was produced by intraperitoneal injection of alloxan (12 mg/100 g) as described by Weber *et al.*[8] and the animals were used 4 days later. In alloxan diabetic rats blood sugar levels were found to be 450–550 mg per cent. Insulin–zinc–protamine (16 U/100g rat) was administered to all of the groups of rats except one group of normal and one group of diabetic rats and all the rats were killed 5 hr later. Actinomycin D (100 μg/100 g body wt.) or cycloheximide (50 μg/100 g body wt.) were also injected into the rats 30 min before the insulin was administered. The animals were killed and the livers and kidneys were removed and stored at $-4°$ for the biochemical studies.

Analytical procedure. Incubation medium for studying the biosynthesis of L-ascorbic acid from L-gulonolactone was carried out according to Chatterjee *et al.*[9]

Details of the other methods for the determination of uronolactonase activity, of the incubation media for studying the synthesis of L-xylulose and catabolism of L-ascorbic acid and estimation of protein were the same as described by Mukherjee *et al.*[4] Blood sugar content was determined by the method of Nelson.[18]

Enzyme source. A portion of liver tissue was taken the blood was removed using blotting paper, weighed and homogenized with 4 vol. of cold isotonic (0·15 M) KCl solution in an all glass Potter–Elvehjem homogenizer for 2–3 min at 4°. Kidney homogenate was prepared in a similar manner, but in this case 3 vol. of 0·15 M KCl was used. All the homogenates were then centrifuged at 10,000 *g* for 40 min at 4°. The supernatants were used as enzyme sources. Liver enzyme was used for the assay of gulonooxidase, dehydroascorbatase and uronolactonase. Kidney enzyme was used for studying the biosynthesis of L-xylulose from L-gulonate.

RESULTS

It appears from Table 1 that the activity of the enzyme "gulonooxidase" which converts L-gulonolactone to L-ascorbic acid is significantly decreased by alloxan treatment. When the alloxan treated animals are subsequently treated with insulin the activity is very strikingly stimulated and this stimulation is not affected by actinomycin D. Cycloheximide treatment, however, markedly diminishes the stimulation to the untreated range. While studying "dehydroascorbatase", the enzyme catalysing the oxidation of dehydroascorbic acid, it was noted that alloxan treatment increased the activity but subsequent administration of insulin alone or insulin along with either actinomycin D or cycloheximide does not show any change. Uronolactonase activity which hydrolyses the lactone to its corresponding free acid was not significantly altered by alloxan treatment. Subsequent administration of insulin along with either actinomycin D or cycloheximide also did not alter the activity of the enzyme. The enzyme responsible for the conversion of L-gulonic acid to L-xylulose was not affected under any of the above described conditions.

TABLE 1. EFFECT OF INSULIN ON THE METABOLISM OF L-ASCORBIC ACID IN RATS

Treatments	Gulonooxidase (nmoles ascorbic acid synthesized/mg of protein)	Dehydroascorbatase (μmoles of 2:3 dioxogulonic acid formed/mg of protein)	Uronolactonase (μmoles D-glucuronolactone hydrolysed/mg of protein)	Gulonate dehydrogenase and gulonate decarboxylase (μmoles of L-xylulose formed/mg of protein)
None	11·49 ± 1·8	0·6476 ± 0·025	1·338 ± 0·137	0·4174 ± 0·074
Alloxan	6·922 ± 1·7†	0·8433 ± 0·161*	1·5732 ± 0·373	0·3838 ± 0.032
Alloxan + insulin	23·17 ± 2·3‡	0·8601 ± 0·319*	1·3801 ± 0·297	0·3928 ± 0·075
Alloxan + insulin + actinomycin D	19·70 ± 0·9‡	0·8740 ± 0·278	1·4660 ± 0·308*	0·4284 ± 0·007
Alloxan + insulin + cycloheximide	12·82 ± 0·8	0·8745 ± 0·111*	1·5278 ± 0·197	0·4266 ± 0·100

Each result is expressed as the mean ±S.D. of four experiments each on a different animal. Details of the experiments are given in Materials and Methods.

*† Mean values significantly different from the control groups of animals (P-values < 0·01 and < 0·05 respectively).

‡ Mean values different from the control group of animals very significant (*t*-value > 5·96 for 6 degrees of freedom).

DISCUSSION

It has long been known that scurvy brings about a status of hypoinsulinism in guinea pigs affecting glycogen level in the liver and skeletal muscles and that prolonged administration of insulin to scorbutic animals improved the glucose tolerance values and the glycogen in the liver and skeletal muscles.[11,12] Insulin injection has also been found to restore the levels of citric acid and ascorbic acid in blood to normal in scorbutic guinea pigs.[13] Recent studies indicate that hormones regulate the synthesis *in situ* of certain hepatic enzymes by influencing the metabolic processes at both the transcriptional and translational events.[14,15] The results presented here indicate that a single injection of insulin to the alloxan treated animals results in an increase in the enzyme gulonooxidase activity. Subsequent administration of actinomycin D does not have any significant effect on enzyme activity while cycloheximide treatment decreases activity to almost the normal level. The enzyme gulonooxidase which is in the pathway of biogenesis of L-ascorbic acid in animals, appears to be the key enzyme, since the enzyme has been found to control the metabolic characteristics of animals with respect to their ability to synthesize L-ascorbic acid. The results indicate that the enzyme gulonooxidase is inducible by insulin and that the synthesis is regulated at the post-transcriptional level. Similar observations with respect to the mechanism of action of insulin have been previously noted in the case of tyrosine transaminase in intact animals[15] and also in cultured hepatoma cells.[16,17] The other enzymes involved in the metabolism of L-ascorbic acid in rats are not greatly affected.

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