Biochemical Pharmacology, Vol. 22, pp. 1829–1833. Pergamon Press, 1973. Printed in Great Britain.

EFFECT OF HYDROCORTISONE ON THE METABOLISM OF L-ASCORBIC ACID IN RATS

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(*Received* 25 *July* 1972; *accepted* 16 *February* 1973)

Abstract—Adrenalectomy causes a significant decrease in the tissue levels of L-ascorbic acid. The ascorbic acid concentration is raised when adrenalectomized animals receive hydrocortisone. Various enzymes of L-ascorbic acid metabolism have been measured in rats after adrenalectomy and subsequent treatment with hydrocortisone and/or actinomycin D. Hydrocortisone stimulates the activity of liver gulono-oxidase responsible for the synthesis of L-ascorbic acid from L-gulonolactone. A slight increase in the activity of dehydroascorbatase is also observed in the adrenalectomized rats. Little change was observed in uronolactonase, L-gulonate dehydrogenase and L-gulonate decarboxylase activities.

INTRODUCTION

THE BIOCHEMICAL role of several hormones has been studied recently at the molecular level and it is known that hormones are involved in enzyme regulation. Hormones such as hydrocortisone, glucagon, insulin, etc. affect hepatic enzyme synthesis especially after adrenalectomy, hypophysectomy and diabetes.[1–4] Adrenal gland contains a very high concentration of ascorbic acid and this is altered to a great extent under stress conditions including that of scurvy in guinea-pigs.[5] It is also known that urinary excretion of L-ascorbic acid is lowered in adrenalectomized and hypophysectomized[6,7] animals and that it plays an important role in the processes of corticosteroids metabolism in animals.[5] Recently studies on the enzymes involved in the metabolism of L-ascorbic acid in animals have been carried out in this laboratory after starvation and subsequent repletion of diet,[8] administration of metal ions[9,10] and variation of quality and quantity of dietary proteins.[11–13] The present communication is concerned with the effect of adrenalectomy and subsequent administration of hydrocortisone and/or actinomycin D to rats *in vivo* in relation to the enzymes involved in the metabolism of L-ascorbic acid.

MATERIALS AND METHODS

Material used. Actinomycin D was a gift from Merck, Sharp and Dohme, U.S.A. Hydrocortisone acetate and L-gulonolactone were purchased from Sigma Chemical Co. and Nutritional Biochemical Corporation (U.S.A.) respectively.

Assay systems. Incubation methods for studying the biosynthesis of L-ascorbic acid from L-gulonolactone are described by Chatterjee.[14] Details of methods for the determination of total ascorbic acid in tissues and for the preparation of rat liver and kidney homogenates, of the incubation media for studying the synthesis of L-xylulose, of the test system to study the catabolism of L-ascorbic acid and of the methods for the determination of uronolactonase activity and protein are described by Mukherjee

et al.[11] After homogenization of livers and kidneys in 0·15 M potassium chloride in an all glass Potter–Elvehjem homogenizer, the homogenates were centrifuged at 10,000 rev/min for 40 min in a refrigerated International centrifuge (Model HR 1). The supernatants have been used as the enzyme source.

TABLE 1. THE EFFECT OF SHAM-OPERATION ON TISSUE LEVELS OF ASCORBIC ACID AND GULONO-OXIDASE ACTIVITY IN LIVER

	Tissue level of ascorbic acid (mg/100 g)			Liver gulono-oxidase (n moles ascorbic acid synthesized/ mg of protein)
	Liver	Kidney	Spleen	
Normal control	34·25 ± 1·12	23·42 ± 0·52	51·54 ± 2·97	10·29 ± 1·02
Sham-operated control	32·63 ± 0·73*	19·91 ± 1·15†	45·83 ± 2·62*	9·94 ± 0·82‡

Mean value ± S.D. of four experimental animals.
* Significantly different from control (P values < 0·05, † P values < 0·01).
‡ Not significant.

Animal experiments. Male albino rats (100–110 g) which had been maintained on a stock diet (10) were divided into six groups, each containing 6–10 rats. The rats in three of these groups were adrenalectomized. These animals were maintained on the same stock diet for 72 hr together with a 1 per cent saline solution *ad lib.* After 72 hr, two groups of normal and two groups of adrenalectomized rats were injected intraperitoneally with hydrocortisone acetate (4 mg/100 g of body wt) for 3 days. On the third day one group of hydrocortisone-treated normal rats and one group of

TABLE 2. EFFECT OF HYDROCORTISONE ON THE TISSUE CONCENTRATION OF ASCORBIC ACID IN RATS*

Treatment	Concentration of ascorbic acid in tissue (mg/100 g)		
	Liver	Kidney	Spleen
None	35·34 ± 0·83	24·83 ± 0·42	54·53 ± 3·87
Hydrocortisone	31·22 ± 0·67‡	26·54 ± 1·01†	46·12 ± 2·59†
Hydrocortisone + actinomycin D	32·32 ± 1·02‡	22·42 ± 0·56‡	44·36 ± 2·15‡
Adrenalectomy	24·09 ± 0·97‡	16·47 ± 0·95‡	33·98 ± 2·47‡
Adrenalectomy + hydrocortisone	31·98 ± 0·65‡	24·22 ± 1·27	39·56 ± 2·02‡
Adrenalectomy + hydrocortisone + actinomycin D	33·54 ± 2·06	21·80 ± 1·19‡	33·75 ± 2·13‡

* Each result is expressed as mean value ± S.D. of six experiments each on a different animal. R. A. Fisher and F. Yates, *Statistical Tables for Biological Agricultural and Medical Research 1953.* Oliver & Boyd Ltd., Edinburgh (1953). Details of the experiments are given in Methods section.
† Mean values significantly different from the control group of animals (P values < 0·001).
‡ Mean values different from the control group of animals very significantly (*t* values > 4·59 for 10 degrees of freedom; also P < 0·001).

adrenalectomized rats treated with hydrocortisone were given a single intramuscular injection of actinomycin D (100 μg/100 g of body wt) 30 min before the administration of the last dose of hydrocortisone. On the third day of treatment the rats were sacrificed 6 hr after hydrocortisone administration. The liver, kidney and spleen were removed. In order to see if adrenalectomy had any effects on ascorbic acid metabolism a sham-operated control group was included. There is no significant alteration in gulono-oxidase activity in the sham-operated group when compared with the controls but the tissue levels of ascorbic acid decreased significantly in the sham-operated groups (Table 1). The extent of this decrease is more pronounced in adrenalectomy (Table 2).

RESULTS

Tissue concentration of ascorbic acid. Adrenalectomy causes a significant decrease in the tissue levels of L-ascorbic acid. Hydrocortisone administration to untreated animals causes a slight decrease in the tissue levels but there is no change after subsequent treatment of the animals with actinomycin D. In the adrenalectomized hydrocortisone-treated animals there is a marked elevation of the tissue levels when compared with the levels in the adrenalectomized animals and this increase is little affected by actinomycin D treatment (Table 2).

Enzymes involved in the metabolism of L-*ascorbic acid.* The activity of the liver gulono-oxidase responsible for the synthesis of L-ascorbic acid from L-gulonolactone decreased significantly in adrenalectomized rats. Administration of hydrocortisone to both the untreated and the adrenalectomized rats, increased the enzyme activity, which could be reduced to the basal level by *in vivo* pretreatment with actinomycin D. The activity of dehydroascorbatase, the liver enzyme involved in the conversion of dehydroascorbic acid to 2:3 dioxogulonic acid, was increased in the adrenalectomized animals but treatment with hydrocortisone alone or with actinomycin D had no significant effect. The activity of liver uronolactonase, catalysing the hydrolysis of D-glucuronolactone to its free acid was significantly decreased by hydrocortisone treatment. Adrenalectomy did not alter the activity of this enzyme. Administration of hydrocortisone alone or with actinomycin D increased the activity of kidney L-gulonate dehydrogenase and L-gulonate decarboxylase responsible for the synthesis of L-xylulose from L-gulonate compared to the controls. Adrenalectomized animals had a lower enzyme activity in comparison to the untreated animals (Table 3).

DISCUSSION

It is evident that the control of enzyme synthesis in animals is mediated through hormones and other metabolic regulators.[2] Some hormones regulate the metabolic processes at the genetic level by influencing synthesis and degradation of enzyme protein.[3] The results from this investigation show that hydrocortisone affects the *in vivo* regulation of gulono-oxidase which is responsible for the synthesis of L-ascorbic acid in animal systems. Adrenalectomy causes a drastic reduction in the activity of this enzyme and subsequent treatment of the animal with hydrocortisone leads to a significant increase in the activity of the enzyme. A similar effect is seen after hydrocortisone administration to untreated animals. Injection of actinomycin D is effective in reversing the stimulatory effect of hydrocortisone and it reduces the

TABLE 3. EFFECTS OF HYDROCORTISONE ON THE ENZYMES INVOLVED IN THE METABOLISM OF L-ASCORBIC ACID IN RATS*

Treatment	Liver Gulono-oxidase (nmoles ascorbic acid synthesized/mg of protein)	Liver Dehydroascorbatase (μmoles of 2:3 dioxogulonic acid formed/mg of protein)	Liver Uronolactonase (μmole D-glucuronolactone hydrolysed/mg of protein)	Kidney Gulonate dehydrogenase and gulonate decarboxylase (μmoles of L-xylulose formed/mg of protein)
None	13·96 ± 0·80	0·64 ± 0·09	1·81 ± 0·18	0·51 ± 0·06
Hydrocortisone	21·76 ± 0·92¶	0·56 ± 0·05	1·53 ± 0·11‡	0·60 ± 0·05‡
Hydrocortisone + actinomycin D	14·11 ± 1·51‡	0·66 ± 0·07	1·60 ± 0·09†	0·58 ± 0·08†
Adrenalectomy	9·71 ± 0·93¶	0·70 ± 0·06§	1·74 ± 0·09	0·45 ± 0·01§
Adrenalectomy + hydrocortisone	19·28 ± 1·17¶	0·58 ± 0·06	1·66 ± 0·02§	0·54 ± 0·04
Adrenalectomy + hydrocortisone + actinomycin D	12·03 ± 0·91¶	0·62 ± 0·08	1·71 ± 0·12	0·56 ± 0·07

* Each result is expressed as the mean ± S.D. of six experiments, each on a different animal. Details of the experiments are given in Methods section.
† Mean values are significantly different from control groups of animals (P values < 0.001, ‡ $P < 0.02$ and § $P < 0.05$ respectively).
¶ Mean values differ significantly from the control group of animals (t value > 4.59 for 10 degrees of freedom, also $P < 0.001$).

enzyme activity to the basal level. Actinomycin D reverses the stimulation of gulonooxidase activity *in vivo* under the influence of hydrocortisone. Other workers have found that its role in enzyme regulation may be due to its effect on enzyme synthesis.[2,3,15] After adrenalectomy L-ascorbic acid synthesis *in vivo* is reduced and this is reflected in the lower tissue levels of ascorbic acid under this condition. The activities of uronolactonase, L-gulonate dehydrogenase and L-gulonate decarboxylase have also been determined but these do not change under the experimental conditions.

Acknowledgement—This research has been financed in part by grant No. FG-IN-467, A7-HN-26 made by the U.S. Department of Agriculture under Public Law 480. The authors thank Professor J. J. Ghosh for his interest and the facilities offered, and Merck Sharp and Dohme for a kind gift of actinomycin D.

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