Biochemical Pharmacology, 1966, Vol. 15, pp. 953–957. Pergamon Press Ltd., Printed in Great Britain.

INFLUENCE OF SUPRARENAL GLAND ON FORMATION OF LIVER GLYCOGEN BY ALANINE*

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(*Received* 1 *October* 1965; *accepted* 12 *January* 1966)

Abstract—Experiments on normal and adrenalectomized albino rats were performed to investigate the influence of the suprarenal gland on the formation of liver glycogen after ingestion of L-alanine. Adrenalectomy greatly reduced glycogen accumulation in the liver, an effect which could be corrected to a large extent if the adrenalectomized animals fed with alanine were injected with cortisol (3 mg/100 body wt.). Subcutaneous injections of adrenaline (0·02 mg/100 g body wt.) did not cause any significant change in liver glycogen of adrenalectomized rats fed with alanine. It is concluded that the suprarenal medulla hormone, adrenaline had no direct influence on this process. The possible mechanisms of action by which the suprarenal cortex hormones influence glycogen formation from alanine are discussed.

THE studies of Long[1] demonstrated that the administration of adrenocortical hormone increases body-carbohydrate stores. The rise in liver glycogen and blood sugar were attributed to gluconeogenesis. Glucocorticoids also inhibit utilization of carbohydrate, and it has been suggested that this is more important than gluconeogenesis in elevating carbohydrate levels.[2, 3] Hess and Shaffran[4] found that cortisone did not increase the production of liver glycogen after feeding glycine, above the additive effect of the individual action of the two compounds. Alanine has been reported to form liver glycogen more rapidly than glycine.[5] Haynes and Okuno recently demonstrated that adrenal steroids stimulate carbohydrate synthesis from alanine when incubated *in vitro* with rat liver,[6, 7] findings which support the idea that a primary effect of these hormones is to enhance glucogenesis. Later on Eisenstein *et al.*,[8] obtained similar experimental results *in vitro* on normal and adrenalectomized animals. Since both studies were conducted *in vitro* using liver slices, and the influence of adrenal hormones *in vivo* has not been thoroughly evaluated, the experiments reported here were carried out. Liver glycogen and adrenal ascorbic acid of normal fasting rats before and after alanine ingestion were investigated. In order to assess the influence of adrenal hormones some experiments were performed on adrenalectomized animals. In addition, since marked differences in the response of those animals was observed, the influence of adrenal hormones in correcting this difference was also estimated.

METHODS

Albino rats approximately 4 months old and weighing 150–200 g were used in the experiments. The rats, fasted for 24 hr before the experiment, were fed the amino

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acid L-alanine by a stomach tube. Alanine dissolved in water, was administered at level of 400 mg/100 g body wt. and a same volume of distilled water was administered in control animals.

For glycogen estimations, the animals were sacrificed by a blow on the head at 2, 4, 6 and 8 hr after ingestion of alanine and in each series an untreated group was also killed and examined. The liver was removed for the determination of glycogen content. Glycogen was estimated after hydrolysis by the method of Good *et al.*[9] Extraction of the suprarenal gland for determination of ascorbic acid content was done by the method described by Nasmyth.[10] Ascorbic acid content of each extract was then estimated by the method described by Barakat *et al.*[11] For purposes of comparison, each value was calculated in terms of mg ascorbic acid/100 g gland tissue. The bilaterally adrenalectomized rats were used 8 days after operation and they received 1% NaCl as drinking fluid after operation.

The following groups of rats were used:

(1) fasted normal rats;
(2) fasted adrenalectomized rats;
(3) fasted normal and adrenalectomized rats injected with adrenaline; and
(4) fasted normal and adrenalectomized rats injected with cortisol.

RESULTS

Effect of alanine ingestion on liver glycogen and adrenal ascorbic acid of normal rats

The results are given in Table 1. The mean liver glycogen concentrations were definitely higher in the alanine-fed animals than the control animals. The maximal

TABLE 1. EFFECT OF ALANINE INGESTION ON LIVER GLYCOGEN AND ADRENAL ASCORBIC ACID OF FASTED MALE RATS

Time after alanine (hr)	Liver glycogen (mg/g)		Adrenal ascorbic acid (mg/100 mg)	
	Control (5)	Alanine treated (6)	Control (5)	Alanine treated (6)
0	3·2 ± 1·4	—	320 ± 11	—
2	3·8 ± 1·3	9·2 ± 2·1	311 ± 17	246 ± 22
4	4·6 ± 1·2	18·2 ± 2·2	328 ± 14	206 ± 35
6	3·3 ± 1·7	29·5 ± 3·1	308 ± 29	178 ± 33
8	3·2 ± 1·2	13·3 ± 2·5	332 ± 21	172 ± 27

Values are means ± S.E. Number of rats in parenthesis. DL-alanine (Merck) administered 400 mg/100 g body wt.

effect was reached after 6 hr. A diminution of ascorbic acid content of the adrenal gland was also observed and the maximum effect was also attained 6 hr after ingestion of the aminoacid.

Effect of alanine ingestion on liver glycogen in adrenalectomized rats

The results are given in Table 2. Adrenalectomy caused a definite lowering of the glycogen content of the liver but there was no significant change in liver glycogen of the adrenalectomized animals after feeding with alanine.

Effect of adrelanine injection on glycogen formation caused by alanine ingestion in the adrenalectomized animal

In both normal fasted animals and adrenalectomized animals fed with distilled water instead of alanine, the subcutaneous injection of adrenaline (0·02 mg/100 g body wt.) first caused a decrease and then a slight increase in liver glycogen. When the

TABLE 2. EFFECT OF ALANINE INGESTION ON FORMATION OF LIVER GLYCOGEN IN ADRENALECTOMIZED RATS

Time after alanine (hr)	Liver glycogen (mg/g)	
	Control (5)	Alanine treated (6)
0	2·1 ± 0·9	—
4	2·3 ± 1·2	2·4 ± 1·1
6	3·2 ± 1·5	3·8 ± 1·6

Values are means ± S.E. Number of rats in parenthesis. DL-alanine (Merck) administered 400 mg/100 g body wt.

same dose of adrenaline was injected in alanine-fed animals, accumulation of glycogen in the liver was observed. Yet, the data in Table 3 shows that such injection into adrenalectomized rats fed with alanine caused practically no change in liver glycogen.

Effect of cortisol injection on glycogen formation caused by alanine ingestion in adrenalectomized animals

The intramuscular injection of cortisol (3 mg/100 g body wt.) led to a normal accumulation of liver glycogen both in the normal and adrenalectomized animals fed with distilled water instead of alanine. When alanine was injected a marked increase in the liver glycogen content could be observed (Table 4).

DISCUSSION

These studies showed that alanine is definitely glyconeogenic in normal albino rats fasted before experiment. It gives a highest value for liver glycogen after 6 hr. It is readily understood how alanine can be converted into glycogen, for deaminated alanine is pyruvic acid, and pyruvic acid can be readily synthesized into glycogen by the liver. The diminution of ascorbic acid content of the adrenal glands after alanine administration, is suggestive of stimulation of the suprarenal cortex.

It is well known that adrenal corticoids promote glyconeogenesis with consequent increased liver glycogen storage. Hess and Shaffran[4] found that cortisone did not cause an increase of liver glycogen in glycine-fed animals, while in this study when using alanine-fed rats a marked increase of liver glycogen could be obtained by cortisol. This may be evidence that alanine is more glyconeogenic than glycine.

Alanine ingestion failed to cause an increase in the liver glycogen of the adrenalectomized animal. It would appear that in the absence of the adrenal cortical hormones a much larger proportion of the alanine absorbed is utilized in other pathways. These might include an accelerated rate of oxidation, or an increased rate of

TABLE 3. EFFECT OF ALANINE INGESTION ON FORMATION OF LIVER GLYCOGEN IN NORMAL AND ADRENALECTOMIZED RATS INJECTED WITH ADRENALINE (0·02 mg/100 g BODY WT.)

Time after ingestion (hr)	Liver glycogen (mg/g)					
	Normal animals (3)	Normal animals + adrenaline (3)	Normal + alanine + adrenaline (4)	Adrenalectomized animals (3)	Adrenalectomy + adrenaline (4)	Adrenalectomy + alanine + adrenaline (5)
0	42· ± 1·2	—	—	2·3 ± 1·1	—	—
2	4·3 ± 1·5	1·9 ± 0·8	18·6 ± 2·3	2·1 ± 1·2	1·6 ± 0·4	2·4 ± 0·9
4	3·8 ± 1·4	1·6 ± 0·6	24·3 ± 3·4	2·1 ± 1·5	6·2 ± 1·2	5·3 ± 1·6
6	2·8 ± 1·1	8·4 ± 1·8	22·6 ± 4·1	1·8 ± 0·8	9·5 ± 1·8	8·2 ± 1·6

Values are means ± S.E. Number of rats in parenthesis. L-alanine administered 400 mg/100 g body wt.

TABLE 4. EFFECT OF ALANINE INGESTION ON FORMATION OF LIVER GLYCOGEN IN NORMAL AND ADRENALECTOMIZED RATS INJECTED WITH CORTISOL (3 mg/100 g BODY WT.)

Time after injestion (hr)	Liver glycogen (mg/g)					
	Normal animals (3)	Normal + cortisol (4)	Normal + cortisol + alanine (5)	Adrenalectomized animals (4)	Adrenalectomy + cortisol (4)	Adrenalectomy + alanine cortisol (4)
0	3·2 ± 1·2	—	—	2·0 ± 1·2	—	—
2	4·3 ± 1·6	9·2 ± 1·8	16·8 ± 2·4	2·2 ± 1·2	6·2 ± 1·4	15·6 ± 2·2
4	3·8 ± 1·4	12·4 ± 2·2	22·8 ± 3·6	1·6 ± 0·8	8·5 ± 1·8	18·8 ± 3·6
6	3·6 ± 1·2	11·6 ± 1·8	36·4 ± 5·2	1·8 ± 0·6	8·4 ± 1·6	23·6 ± 4·2

Values are means ± S.E. Number of rats in parenthesis. L-alanine administered 400 mg/100 g body wt.

fatty acid synthesis. Welt and Wilhelmi[12] have brought forward some evidence which indicates that the rate of fatty acid synthesis is increased in adrenalectomized rats. Kerpolla[13] has found that large doses of cortisone given to rabbits greatly reduce the phosphorylase activity of liver and muscle; leading, in his view, to an accumulation of glycogen in consequence of the inhibition of glycogenolysis. If this is correct, then in the absence of cortical hormones an increased rate of glycogenolysis would presumably occur. Rosen *et al.*,[14] studied the activity of glutamic–pyruvic transaminase in the liver of rats in some conditions known to be associated with enhanced gluconeogenic activity and they found a 5 to 7-fold increases in liver glutamic–pyruvic transaminase activity occurred in rats fed with high protein rations. This could account for the rise in liver glycogen of alanine fed rats.

This study also showed that the suprarenal medulla hormone, adrenaline, has nothing to do with the accumulation of glycogen in the liver caused by alanine ingestion. It is well known that adrenaline in the intact animal at first depletes and then increases liver glycogen along with its hyperglycemic effect.[15] Since it is also known that adrenaline injection provokes a secretion of adrenal cortical hormones,[16] it is suggested that at least a part of the normal response of the carbohydrate metabolism of fasted rats to injection of adrenaline is a consequence of the concomittant effect of this hormone in stimulating the release of ACTH from the anterior lobe of the pituitary gland.

As the injection of Cortisol in the adrenalectomized animal was able to restore the carbohydrate balance to that found in the intact rat, it would appear that there is a stimulation of glycogen formation by suprarenal cortex when alanine is fed.

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