The Mechanism by which Administration of Individual Amino Acids causes Changes in the Ribonucleic Acid Metabolism of the Liver

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The influence of individual dietary amino acids on RNA metabolism in the liver was examined in an earlier series of experiments (Munro & Mukerji, 1958). Eighteen amino acids were fed singly in large doses and it was established that glycine, methionine and leucine each caused a considerable increase in uptake of ^{32}P by liver RNA, accompanied by a small but significant increment in the total amount of RNA in the liver. From the available evidence it was concluded that the action of these amino acids on RNA metabolism was secondary to an increased rate of protein synthesis in the liver, and it was suggested that this might be due to stimulation of the secretory activity of the adrenal cortex by the excessive intake of amino acids. This possibility has been examined in the present series of experiments, and evidence has been obtained that the action of methionine and of leucine is dependent on adrenocortical-hormone secretion. A preliminary account of these experiments has been published (Munro & Mukerji, 1960).

METHODS

The procedures were similar to those used previously (Munro & Mukerji, 1958).

Animals and diets. Male albino rats weighing 180–200 g. were housed individually and fed on a synthetic diet (Munro & Naismith, 1953) providing adequate amounts of protein. The diet was given in two meals; the morning portion contained vitamins, minerals, 4.3 g. of carbohydrate and 0.2 g. of fat, and the evening meal contained 1.3 g. of carbohydrate, 0.5 g. of fat, and the protein of the diet, 2.2 g. of casein. As soon as the rats had learned to consume these meals promptly, the test protein or amino acid was substituted for the protein of the evening meal; control animals received only the carbohydrate and fat of this meal. After 1 hr., $40\text{ }\mu\text{C}$ of ortho[^{32}P]phosphate was injected intramuscularly. The rats were killed the next morning, 18 hr. after injection of the isotope.

Removal and analysis of the liver. The livers were removed under ether anaesthesia and analysed for nucleic acid phosphorus (RNA P and DNA P) by methods described earlier (Clark, Naismith & Munro, 1957). The amount of RNA P was expressed as mg./mg. of DNA P, with DNA as a standard of reference since it is not influenced by dietary treatments (Thomson, Heagy, Hutchison & Davidson, 1953).

The specific activities of the inorganic phosphate of the liver and of the individual ribonucleotides of liver RNA

were measured by procedures described previously (Clark *et al.* 1957). These specific-activity data were used to compute the relative specific activity of the RNA P (specific activity of RNA P as a percentage of the specific activity of the inorganic phosphate fraction of the same liver). In order to estimate the total incorporation into RNA P per liver, the total relative activity (Campbell, Olley & Blewett, 1949) was obtained by multiplying the total amount of liver RNA P in mg./mg. of DNA P by the percentage figure obtained for relative specific activity.

The glycogen content of the liver was measured by a procedure described previously (Munro, Clark & Goodlad, 1961). The total amount of glycogen in the liver has been expressed as mg./100 g. body wt., to allow for differences in size of rat used.

Test protein and amino acid. The final meal contained either 1 g. of an amino acid or 2.2 g. of casein. The casein was unextracted grade (Glaxo Laboratories Ltd., Greenford, Middlesex). The amino acids were commercial preparations (British Drug Houses, Poole, Dorset). The glycine was of AnalaR quality; the L-leucine was stated to contain traces of L-isoleucine.

Administration of cortisone. In one series of experiments, instead of feeding an amino acid in the final meal, cortisone acetate (Upjohn of England Ltd., London) was administered at the time of giving the final meal of carbohydrate and fat. Cortisone acetate (5 mg./100 g. body wt.) was suspended in 0.2 ml. of aq. (0.9%) NaCl and injected subcutaneously. Control animals received a similar vol. of 0.9% NaCl. After 1 hr., $40\text{ }\mu\text{C}$ of ^{32}P was given intramuscularly, and the animals were killed 18 hr. later.

Adrenalectomy. The rats were bilaterally adrenalectomized by the dorsal route. 'Sham-operated' control animals were subjected to the same incision without removal of the adrenal glands. All adrenalectomized animals were maintained on aq. (1%) NaCl in place of drinking water. The feeding of amino acids was performed about 10 days after operation.

RESULTS

In our earlier paper (Munro & Mukerji, 1958) it was shown that the feeding of large amounts of individual amino acids could increase uptake of ^{32}P by liver RNA, and we suggested that this action might be due to stimulation of the secretory activity of the adrenal cortex by the individual amino acids. A characteristic feature of increased adrenocortical activity is the deposition of glycogen in the liver. An experiment was therefore carried out in order to determine whether the feeding of

large amounts of glycine, methionine or leucine gives rise to accumulation of glycogen in the liver. These amino acids were given in 1 g. quantities to rats in a meal of carbohydrate and fat, and the total glycogen content of the liver was measured 18 hr. later. At this time after feeding, control animals receiving only the carbohydrate and fat components of the meal had only small quantities of glycogen in their livers (Table 1). Rats receiving the amino acids had considerably larger amounts of liver glycogen, the effect of amino acid administration being shown by analysis of variance to be statistically significant ($P < 0.05$). A similar experiment was then performed with rats which had been adrenalectomized some 10 days beforehand. When the same amino acids were administered to this group of rats, no deposition of liver glycogen was observed (Table 1). From these findings it would appear probable that adrenocortical hormone activity can be increased by amino acid administration.

Administration of cortisone has been reported by Silber & Porter (1953) and by Goodlad & Munro (1959) to increase the RNA content of the

livers of rats, but Lowe & Rand (1956) did not obtain any appreciable increment in liver RNA with much larger quantities of the hormone. In order to determine whether, under the conditions used for amino acid administration, liver-RNA metabolism is influenced by adrenocortical hormone activity, we carried out an experiment in which cortisone was given at the time of feeding the final meal of carbohydrate and fat used as a vehicle for the amino acids. Table 2 shows that this resulted in increments in the amount of RNA in the liver and in the incorporation of ^{32}P into liver RNA; both of these actions of cortisone were shown to be statistically significant by the t test ($P < 0.05$).

Finally, experiments were carried out in which bilaterally adrenalectomized rats were fed with glycine, methionine or leucine and the action of these amino acids on liver-RNA metabolism was examined. Table 3 shows that the 'sham-operated' control animals responded to amino acid administration in the manner previously established with normal rats (Munro & Mukerji, 1958). In each case, the giving of the amino acid caused a significant increase in ^{32}P uptake by liver RNA. In the adrenalectomized rats, no stimulus to RNA metabolism was obtained after administration of methionine or leucine; this lack of response is significantly different from the changes observed in the 'sham-operated' animals after feeding these two amino acids. Glycine continued to evoke a significant increase in ^{32}P uptake when given to adrenalectomized animals; the magnitude of the stimulus appeared to be less than in the corresponding 'sham-operated' group, but this could not be established by statistical analysis. Animals fed with casein in the final meal were also included in these experiments; this increased uptake of ^{32}P into RNA in the livers of both the 'sham-operated' and the adrenalectomized rats, the effect being statistically indistinguishable in the two groups.

DISCUSSION

Table 1. *Glycogen content of liver after administration of individual amino acids to rats*

The rats received a final meal of carbohydrate and fat to which was added 1 g. of glycine, L-methionine or L-leucine. They were killed 18 hr. later. Adrenalectomized animals were used about 10 days after operation. Each entry is the mean value, with its standard error, for 5 rats in the case of the control group and 3 rats in the case of the adrenalectomized group.

Addition to final meal	Liver glycogen (mg./liver/100 g. body wt.)	
	Control group	Adrenalectomized group
None	8±1	7±1
Glycine	27±6	8±2
Methionine	39±14	5±1
Leucine	16±6	6±1

Table 2. *Liver-ribonucleic acid metabolism after administration of cortisone to rats*

The rats were given a final meal of carbohydrate and fat followed by injection of cortisone acetate suspended in 0.9% NaCl; control animals received 0.9% NaCl. After 1 hr., the animals were injected with 40 μC of ^{32}P and were killed 18 hr. later. Each entry is the mean value, with standard error, for five rats.

Group of rats	RNA P (mg./mg. of DNA P)	Uptake of ^{32}P by RNA (total relative activity)
Control	2.86±0.10	0.92±0.12
Cortisone	3.35±0.15	2.03±0.41

Administration of glycine, methionine or leucine in large amounts to rats causes an increase in uptake of ^{32}P by the RNA of the liver (Munro & Mukerji, 1958). The present experiments establish that intact adrenal glands are necessary for the action of methionine and of leucine on liver-RNA metabolism (Table 3). The evidence points to stimulation of the adrenal cortex by these amino acids. First, administration of cortisone can produce effects on liver-RNA metabolism similar to those observed after feeding these amino acids (Table 2); secondly, it has been shown (Table 1) that glycogen deposition in the liver, a characteristic feature of glucocorticoid activity, occurs after

Table 3. *Liver-ribonucleic acid metabolism after administration of individual amino acids or casein to adrenalectomized and to 'sham-operated' rats*

The rats received a final meal of carbohydrate and fat to which was added 1 g. of glycine, L-methionine or L-leucine, or 2.2 g. of casein. After 1 hr., 40 μ C of 32 P was injected and the rats were killed 18 hr. later. Each entry is the mean value for five animals. The change in uptake of 32 P by RNA due to administration of the amino acid or protein is given with its standard error.

Addition to final meal	Uptake of 32 P by RNA (total relative activity)			
	Total uptake		Change caused by addition to meal	
	'Sham-operated' group	Adrenalectomized group	'Sham-operated' group	Adrenalectomized group
None	0.51	0.59	—	—
Glycine	1.07	0.96	+0.56 $\pm$ 0.06†	+0.37 $\pm$ 0.12*
Methionine	0.97	0.52	+0.46 $\pm$ 0.09†	-0.07 $\pm$ 0.14§
Leucine	0.85	0.60	+0.34 $\pm$ 0.07†	+0.01 $\pm$ 0.07§
Casein	1.10	0.99	+0.59 $\pm$ 0.08†	+0.40 $\pm$ 0.14*

* By the *t* test, the change in 32 P uptake is significant ($P < 0.05$).

† By the *t* test, the change in 32 P uptake is significant ($P < 0.02$).

‡ By the *t* test, the change in 32 P uptake is significant ($P < 0.01$).

§ The change in 32 P uptake does not differ significantly from zero; it is significantly less than the effect of feeding the same amino acid to the 'sham-operated' rats ($P < 0.02$).

giving large amounts of these amino acids to normal rats but not after their administration to adrenalectomized rats. The sequence of events after administration of large amounts of methionine or leucine would thus appear to be an increase in glucocorticoid secretion, followed by deposition of protein and RNA in the liver, which are recognized glucocorticoid effects (Trémolières, Derache & Lowy, 1955). The mechanism of action of glycine on liver-RNA metabolism appears to differ from that of methionine and leucine, since an effect still occurs on feeding glycine to adrenalectomized animals. A difference between the actions of glycine and of methionine and leucine on liver-RNA metabolism was noted previously (Munro & Mukerji, 1958). The responses in RNA metabolism observed after the feeding of methionine and of leucine were shown to be proportional to the dose administered, whereas the response to glycine occurred only after a threshold dose had been reached.

SUMMARY

1. The reason for the increase in 32 P uptake by liver RNA after administration of large amounts of glycine, methionine or leucine to rats was investigated.

2. Normal rats killed 18 hr. after feeding these amino acids showed deposition of glycogen in their livers, whereas adrenalectomized animals did not exhibit this effect.

3. Administration of cortisone to rats caused

the same changes in liver-RNA metabolism as those observed after feeding the amino acids.

4. Adrenalectomized rats no longer showed an increased uptake of 32 P into liver RNA when methionine or leucine was given, but feeding with glycine was still effective.

5. Methionine and leucine produce their action on liver-RNA metabolism through stimulation of adrenocortical activity.

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