The Concentration of Asparagine in the Tissues of Rats Treated with Growth Hormone

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(Received 20 June 1972)

In recent years it has been established that asparagine is a limiting growth factor for certain tumours, for leukaemias and for lymphoid tissue, and it has been found that growth of the tissue can be inhibited in each case by the intravenous injection of asparaginase. In some circumstances, e.g. a low-protein diet, asparagine given intraperitoneally or by mouth can increase the rate of growth of young rats (Breuer *et al.*, 1966). The growth in adult rats induced by growth hormone involves the retention of N and the permanent laying down of new protein, particularly in muscle (Greenbaum & Young, 1953); it seems possible that asparagine may be a limiting factor in this growth also, particularly since it is very unlikely that muscle contains a significant amount of asparagine synthetase. In order to assess the likelihood of this idea, the asparagine concentration in cardiac and skeletal muscle of rats treated with bovine growth hormone has been compared with that of controls. Because there are rather few values for asparagine concentrations in tissues to be found in the literature, the asparagine contents of other visceral organs, of brain and of blood were also measured. The assay method used involved the simultaneous measurement of aspartate, and the concentrations of this amino acid in the tissues chosen are also reported.

It is doubtful whether stringent enough arrangements to arrest metabolism rapidly were made in most determinations of tissue aspartate concentrations that have been published until quite recently. If tissues are allowed to remain in an anaerobic state for more than a few seconds, oxidation of glutamate to aspartate can occur at a rapid rate. To guard against the possibility that the aspartate concentrations reported here had been raised by post-mortem changes the concentration of glutamate was measured in each sample.

Methods

Asparagine was determined as the difference in concentration of aspartate in a tissue extract before and after incubation with L-asparaginase [Boehringer Corp. (London) Ltd., London W.5, U.K.]. Aspartate was measured by coupling the production of oxaloacetate, formed on the addition of glutamate-oxaloacetate transaminase and α -oxoglutarate, to the

oxidation of excess of NADH catalysed by malate dehydrogenase. The method was similar in essentials to that described by Cooney *et al.* (1970). Glutamate was measured by the method of Sowerby & Ottaway (1966).

Adult female albino rats were injected subcutaneously with 1 mg of bovine growth hormone (Calbiochem Ltd., London W.1, U.K.) in saline (0.9% NaCl) per day for 4–5 days. Controls were injected with saline only. The rats were killed 12–18 h after the last injection, by dislocation of the cervical vertebrae; tissues were taken rapidly and dropped into *n*-pentane cooled in liquid N₂. The frozen tissue was crushed in a percussion mortar cooled to –70°C and homogenized in 1 M-HClO₄ in 25% (v/v) ethanol at –10°C. The precipitated protein was spun off and dried to constant weight at 105°C to provide an estimate of the weight of tissue taken. The HClO₄ extract was neutralized in the usual way.

Not more than three organs were taken from any animal, and the order of excision was varied, except that when the brain was taken the entire head was cut off first and dropped into chilled pentane. After the other tissues had been extracted the skull was split open and the brain tissue scooped out with a chilled spatula. Blood was taken by exsanguination at the neck or by cardiac puncture; because Cooney *et al.* (1970) have shown that the concentration of asparagine in erythrocytes is little higher than that of plasma in the rat, whole blood was used for assay.

Results

Table 1 shows that the concentration of asparagine was decreased by 50% in heart and skeletal (leg) muscle after treatment with growth hormone. The concentration in blood also fell significantly and there was a significant but unexpected fall in the concentration in brain. Brain growth is not stimulated by growth hormone (Simpson *et al.*, 1949), so that the fall in concentration in this organ is rather puzzling.

Table 1 shows that asparagine is concentrated with respect to blood, and by implication with respect to plasma (Cooney *et al.*, 1970), in all the tissues assayed, although the concentration ratio is very modest by comparison with that for aspartate (Table 2). The

Table 1. *Effect of treatment with growth hormone on the asparagine content of rat tissues*

The treatment with growth hormone (1 mg/day) or saline was for 4–5 days. Experimental details are given in the text. The results for amino acid content are given as means $\pm$ S.E.M., with numbers of determinations in parentheses. *P* values for the significance of differences between values for control and hormone-treated animals were calculated by using Student's *t* test. The partition ratio is the ratio of the concentration in the tissue fluid (without correction for the extracellular fluid content of the tissue, and assuming a wet weight/dry weight ratio of 6:1) to that in blood.

	Asparagine content ($\mu\text{mol/g dry wt.}$)		Significance	Partition ratio	
	Control	Growth hormone		Control	Growth hormone
Brain	0.33 $\pm$ 0.06 (3)	0.13 $\pm$ 0.03 (4)	$P < 0.05$	1.9	1.0
Liver	0.14 $\pm$ 0.06 (3)	0.14 $\pm$ 0.015 (4)		0.8	1.1
Spleen	0.43 $\pm$ 0.06 (3)	0.34 $\pm$ 0.11 (3)		2.5	2.7
Kidney	0.26 $\pm$ 0.03 (3)	0.30 $\pm$ 0.03 (3)		1.5	2.4
Heart	0.64 $\pm$ 0.04 (3)	0.49 $\pm$ 0.05 (3)	$P = 0.05$	3.7	2.5
Muscle	0.32 $\pm$ 0.01 (4)	0.19 $\pm$ 0.02 (5)	$P = 0.001$	1.9	1.5
Blood	0.0285 $\pm$ 0.003 (4)	0.021 $\pm$ 0.003 (4)	$P < 0.05$		

Table 2. *Effect of treatment with growth hormone on the aspartate content of rat tissues*

For details see the legend to Table 1.

	Aspartate content ($\mu\text{mol/g dry wt.}$)		Significance	Partition ratio	
	Control	Growth hormone		Control	Growth hormone
Brain	6.43 $\pm$ 0.44 (3)	6.25 $\pm$ 0.49 (4)		46.6	61.3
Liver	2.45 $\pm$ 0.35 (4)	2.79 $\pm$ 0.29 (4)		17.7	27.4
Spleen	7.97 $\pm$ 0.40 (3)	7.65 $\pm$ 0.92 (4)		57.7	75.0
Kidney	2.47 $\pm$ 0.15 (3)	2.50 $\pm$ 0.27 (3)		17.9	24.5
Heart	5.03 $\pm$ 0.34 (3)	3.06 $\pm$ 0.77 (3)	$P < 0.02$	36.4	30.0
Muscle	1.07 $\pm$ 0.15 (4)	0.46 $\pm$ 0.06 (5)	$P = 0.005$	7.7	4.5
Blood	0.023 $\pm$ 0.004 (4)	0.017 $\pm$ 0.004 (5)			

aspartate ratios for all tissues, except skeletal muscle, are very similar to those quoted for ascites cells by Christensen *et al.* (1952). These authors also reported that asparagine inhibits transport of glycine and alanine, and of lysine, into ascites cells, and therefore has affinity with the lysine-preferring transport system and with at least one of the two systems for neutral amino acids (Christensen, 1964). It has been suggested (Riggs, 1970) that one of the latter is sensitive to growth hormone and the other is not. If asparagine were transported by a hormone-sensitive system in some tissues and by an insensitive system in others, the curious inhomogeneity of response to growth hormone could be explained. The situation may, however, be more complicated than this. Cooney *et al.* (1970) have pointed out that the asparagine concentration in rat erythrocytes changes only very slowly in response to changes in the plasma concentration. Since the cell/plasma concentration ratio is normally slightly but definitely greater than

1, there must be a transport system for asparagine in erythrocytes; one explanation for the insensitivity to changes in the plasma concentration is that the system facilitates influx but not efflux (cf. Kotyk, 1972). If this suggestion is correct, it could be that the asparagine-transporting systems in kidney and spleen facilitate influx only and therefore do not reflect alterations in the plasma concentration. The fall in concentration in brain and muscle might then be a result of asparagine transport, in these tissues, by an influx-efflux system that responds to changes in the external concentration. It seems likely that in the tissue that increases in weight during treatment with growth hormone, namely skeletal muscle, the fall in asparagine concentration is a result of demand outstripping supply.

It then remains to consider the reason for the fall in blood asparagine concentration as the result of treatment with growth hormone. The possibility that growth hormone inhibits asparagine synthesis

cannot yet be ruled out, but may be discounted. Cooney *et al.* (1970) have shown that the plasma asparagine concentration does not change after hypophysectomy, and it was pointed out above that asparagine can in some circumstances be rate-limiting for growth in rats. It seems most probable that the rate of utilization for the synthesis of new protein during treatment with growth hormone outstrips the rate of formation of the amino acid itself. Asparagine synthetase has been found in liver, and it may be significant that Table 1 shows that only in liver was the partition ratio near unity, a result that one might expect if liver is the main source of new asparagine for other tissues in the body.

Table 2 shows that the concentration of aspartate fell significantly in heart and skeletal muscle; the fall in blood concentration was not statistically significant. The fall in aspartate concentration was not due to inhibition of post-mortem glutamate oxidation, because after the treatment with growth hormone glutamate concentration was unchanged in skeletal muscle and had risen by a far greater amount than the fall in aspartate in heart muscle. Indeed the concentration of glutamate rose, during treatment with growth hormone, by an average of 50% in every organ assayed, with the exception of skeletal muscle. There was a large variance in the values obtained for liver, which indicates that the results for this tissue ought to be re-examined in experiments designed to avoid even the few seconds' delay caused by the excision of more than one organ, but the results for brain, kidney, heart and spleen glutamate were statistically significant.

Although a great deal of work has been done on the effects of growth hormone on N metabolism (Ketterer *et al.*, 1957) and on its effects on the transport of amino acids into tissues, particularly muscle (Riggs, 1970), similar observations on changes in aspartate and glutamate concentrations seem not to have been reported previously. This is partly due to the fact that accurate measurements of concentrations of aspartate and glutamate *in vivo* have only rarely been made until surprisingly recently. This can be assessed by following the steep decline in the reported concentration of tissue aspartate through the years, the values obtained by Herbert *et al.* (1966) being among the first reliable estimates. In previous measurements of the effect of hormone treatment on the concentra-

tions of these two amino acids the effect of post-mortem changes (Krebs, 1950) may well have swamped any genuine differences. The second reason for the apparent oversight is that those who were interested in studying the effect of hormones on amino acid transport naturally avoided those that are rapidly metabolized in the tissues. Little is known of the transport systems for the anionic amino acids. It is in any case quite probable that the observed changes in aspartate and glutamate concentrations were the result of changes in the pattern of intracellular metabolism rather than of transport through the cell membrane. They may, of course, reflect changes in permeability barriers inside the cell, induced by growth hormone, of the kind postulated by Ottaway (1969).

These experiments thus demonstrate that growth hormone does cause changes in asparagine concentration that are compatible with the possibility that this amino acid can be rate-limiting in hormone-induced growth, although much work remains to be done before the results are fully explained.

My thanks are due to Mr. R. A. Croy for developing the asparagine assay method.

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