The Potentiation of Growth Hormone by Asparagine and Tryptophan

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Asparagine potentiates the growth-promoting effect of bovine growth hormone in rats when injected 3 h after the latter, but not earlier. Tryptophan potentiates only when injected simultaneously with the hormone. The possible mode of action of growth hormone, and the likelihood that asparagine is a limiting factor in the growth of animals, are discussed.

It has been reported that the asparagine contents of skeletal muscle, heart, brain and blood of adult female rats fall after injection for 5 days with bovine growth hormone (Ottaway, 1972), and it was suggested that asparagine might be a limiting factor in hormone-induced growth. Frankel *et al.* (1973) found that weanling rats either fed on an asparagine-deficient diet or injected with asparaginase grow less fast than do controls and at the same time the asparagine content of muscle and forebrain is decreased. If asparagine is a limiting factor during rapid growth in rats, supplementation should remove the limitation and allow growth to take place even faster. However, in view of the possibility that another limiting factor might rapidly replace asparagine, so that the increase in growth might only be marginal, and difficult to detect, it was decided to test the hypothesis in a slightly different way, by observing whether asparagine potentiates the effect of suboptimal doses of growth hormone. To guard against the possibility that asparagine might be only one of a number of amino acids present in low concentration in the body free amino acid pool that would all be equally effective, it was decided to compare asparagine with tryptophan (Herbert *et al.*, 1966; Munro, 1970).

For each experiment 24 adult female rats were divided randomly among a $2 \times 2 \times 2$ factorial design with three animals in each block. They were fed *ad lib.* on Oxoid Diet 41B (Oxo Ltd., London S.E.1, U.K.), which contains 15.9% crude protein. The amino acids were injected subcutaneously. The amino acid doses were 10 mg of L-tryptophan in 1 ml of 0.9% NaCl and 10 mg of L-asparagine in 0.4 ml of 0.9% NaCl. Bovine growth hormone [0.5 mg from Calbiochem Ltd. (London W.1, U.K.) in the first experiment and 0.8 mg from Koch–Light Laboratories Ltd. (Colnbrook, Bucks., U.K.) in the second] was also injected subcutaneously in 0.9% NaCl. The animals were injected daily for 6 days. Controls were injected with 0.9% NaCl. Tissue analyses were carried out as described previously (Ottaway, 1972).

All results were treated statistically by analysis of variance of factorial design (Snedecor & Cochran, 1967).

Two experiments were carried out. In the first the amino acids were injected at the same time as the growth hormone, in the late afternoon. The growth rate of the controls was about 1.2 g/day. Neither growth hormone nor asparagine nor tryptophan alone increased the rate of growth above that of the controls, but growth hormone together with tryptophan significantly increased the growth rate (to 1.8 g/day; F -ratio 5.54; $P < 0.05$). Asparagine had no effect when injected together with tryptophan or with growth hormone.

In the second experiment the amino acids were injected 3 h after growth hormone, which was injected at about 10 a.m. Growth hormone by itself was again without effect, but this time asparagine but not tryptophan potentiated its action. The growth rate increased from 1.8 to 2.8 g/day ($F = 4.85$; $P < 0.05$). Tryptophan plus asparagine was no more effective than asparagine alone.

At the end of this experiment the animals were killed 2–4 h after the last amino acid injection. Samples of blood, brain, thigh muscle and kidney were taken from each animal and analysed for aspartate and asparagine as previously described (Ottaway, 1972).

The results of these experiments may be interpreted in terms of a fast effect and a slow effect of growth hormone. The fast effect is that which is potentiated by tryptophan when it is injected concurrently with the hormone. It is tempting to identify this with an effect on the liver, for example a tryptophan-mediated increase in the polyribosome profile of hepatocytes (Munro, 1970), perhaps leading to an increased rate of synthesis of 'somatomedin' (Tanner, 1972). However, it is equally possible that the effect of tryptophan was an indirect result of the increase in brain 5-hydroxytryptamine (serotonin) that follows a rise in plasma tryptophan concentration (Fernstrom & Wurtman, 1971), or the rise in plasma insulin concentration after tryptophan injection

(Floyd *et al.*, 1966). The fact that asparagine did not potentiate the 'fast effect' also argues to some extent against the liver as the primary site on this effect, since Jefferson & Korner (1969) found asparagine, as well as tryptophan (and nine other amino acids) to be effective in maintaining the polyribosome profile of perfused liver.

In analysing the slow effect, i.e. that potentiated by asparagine, but not by tryptophan, when either amino acid is injected 3 h after growth hormone, one may observe first of all that stimulation of growth by growth hormone plus asparagine occurred even though the 'fast effect' had not been potentiated by tryptophan. It is therefore clear that, whatever the result of the 'fast' interaction between tryptophan and growth hormone when they are injected simultaneously, it is not essential that the rapid effect of growth hormone should be elicited at full strength for hormone-induced growth to occur. This strengthens the argument implicit in the previous paragraph, that the effect of tryptophan was to some extent an adventitious one. This is supported by work of Alcock (1934), showing that rats on tryptophan-deficient diets can grow when injected with growth hormone, and also that subcutaneously injected tryptophan does not support the growth of rats on such a diet.

These experiments only tell us, about the 'slow effect' of growth hormone, that it can be potentiated by asparagine at least 3 h after the hormone was injected. It may be argued on general grounds that it is due to the stimulation of some event, or events, in muscle. In these short-term experiments the measured response was weight gain, and effects on bone growth may be neglected. The tissue providing the largest component of the gain in weight in growth-hormone-treated animals is skeletal muscle (Greenbaum & Young, 1950). It has been shown (James, 1972) that protein synthesis in rat muscle is very much enhanced during the post-absorptive period; it may be assumed that this is also true for growth-hormone-induced growth, even though skeletal muscle from normal animals will respond immediately (e.g. *in vitro*; Turner, 1972). Since the post-absorptive period for rats is in the middle of the night, it follows that the major effect of growth hormone in increasing muscle weight must occur many hours after a day-time injection of the hormone.

There is much evidence which suggests that asparagine is a limiting factor in muscle and body weight gain. Asparagine supplements to an otherwise complete diet increase the growth rate of weanling rats (Rogers & Harper, 1965; Breuer *et al.*, 1966), at least on a short-term basis (Crosby, 1972). On the other hand young rats depleted of asparagine grow more slowly than normal (Frankel *et al.*, 1973). The depression of growth is associated with a significant decrease in the asparagine contents of muscle and forebrain, alone, of the tissues investigated. Ottaway

(1972) showed that, 18 h after the last of a series of growth-inducing doses of growth hormone, the concentrations of asparagine in the blood, muscle and brain were significantly lower than in control tissues, and suggested that this showed that the demand for asparagine in the hormone-stimulated muscle was greater than the capacity of the body to manufacture it or to obtain it from food. This is supported by Crosby's (1972) observation that the asparagine synthetase activity in rat liver declines markedly as the animals approach maturity.

The results shown in Table 1 support the view that there is a specific connexion between asparagine, muscle and growth. They show that, 3 h after subcutaneous injection of asparagine, the asparagine concentration is raised in blood and skeletal muscle, but not in kidney or brain (other tissues could not be assayed for technical reasons). Asparagine is the amino acid present in lowest concentration in muscle (Herbert *et al.*, 1966), and the rate of formation of asparaginyl-tRNA may therefore be rate-limiting in the synthesis of muscle protein, in a way comparable with the postulated effect of tryptophan in liver (Munro, 1970). The tryptophan concentration in muscle is much higher than that of asparagine, and supplementation with it might well not potentiate an increase in muscle weight [although one may note a serious discrepancy between the values quoted by Herbert *et al.* (1966) for the experimentally determined tryptophan content of the whole carcass and that which one may calculate to be present from the analyses of individual tissues]. If asparagine is indeed rate-limiting, one would expect that one effect of growth hormone would be to raise the muscle asparagine content soon after injection. In these experiments the effect of growth hormone on muscle asparagine approached but did not reach the level of statistical significance. This correlates well with the effect of the hormone on the growth of the same animals, which was also not statistically significant when asparagine supplements were not given.

There is one objection to this hypothesis, which cannot be resolved without further experimental work. If the potentiating effect of asparagine is due simply to the loading of muscle with the amino acid, one would expect that injection of asparagine at the same time as growth hormone should be almost as effective as injection 3 h afterwards, since the muscle concentrations are elevated for at least 3 h after injection (Table 1); Christensen (1964) implies that muscle is the major reservoir of free amino acids and that the unloading of amino acids to plasma after their accumulation is rather slow.

Asparagine did not potentiate growth hormone when injected simultaneously with it. One possible explanation is that the rise and fall in the concentration of asparagine in muscle is rather rapid, so that it has fallen to normal by the time that the 'slow effect'

Table 1. *Asparagine contents of rat tissues*

The values are the means of three observations for each of the treatments shown. The residual sums of squares, *F*-ratios and significance levels are given at the right-hand side of the table.

		Asparagine content ($\mu\text{mol/g}$ dry wt. of tissue*)				Analysis of variance							
						Interactions							
						Asparagine (1)	Tryptophan (2)	Growth hormone (3)	(1) $\times$ (2)	(1) $\times$ (3)	(2) $\times$ (3)	Residual	
Muscle	Tryptophan ...	-	-	+	+								
	Growth hormone ...	-	+	-	+								
Kidney	Asparagine												
	-	0.106	0.194	0.052	0.159	<i>F</i>	4.87	1.24	1.49	0.08	3.53	1.95	0.006
Kidney	+	0.408	0.711	0.306	0.364	<i>P</i>	<0.05				0.08		—
	-	0.290	0.500	0.225	0.443	<i>F</i>	0.06	0.18	0.95	1.21	1.96	0.29	0.049
Brain	+	0.243	0.304	0.480	0.342	<i>P</i>							—
	-	0.187	0.459	0.446	0.541	<i>F</i>	0.22	4.25	0.47	0.005	2.13	0.08	0.044
Blood*	+	0.330	0.213	0.473	0.446	<i>P</i>		0.06					—
	-	0.023	0.033	0.027	0.062	<i>F</i>	17.3	2.25	0.10	7.15	1.90	1.04	0.0008
Blood*	+	0.120	0.100	0.064	0.063	<i>P</i>	<0.005			<0.025			—
	* See Ottaway (1972).												

Table 2. *Aspartate contents of rat tissues*

The values are the means of three observations for each of the treatments shown. The residual sums of squares, *F*-ratios and significance levels are given at the right-hand side of the table.

		Aspartate content ($\mu\text{mol/g}$ dry wt. of tissue)				Analysis of variance							
						Interactions							
						Asparagine (1)	Tryptophan (2)	Growth hormone (3)	(1) $\times$ (2)	(1) $\times$ (3)	(2) $\times$ (3)	Residual	
Tryptophan	...	-	-	+	+								
Growth hormone	...	-	+	-	+								
Muscle	Asparagine												
	-	0.408	0.520	0.295	0.388	<i>F</i>	0.05	0.08	0.23	1.82	3.40	0.68	0.034
	+	0.409	0.349	0.604	0.314	<i>P</i>				0.085		—	
Kidney	-	2.405	2.211	1.723	3.326	<i>F</i>	1.07		2.44	2.21	0.94	4.77	0.464
	+	3.085	2.933	2.157	2.639	<i>P</i>		0.50				<0.05	
Brain	-	3.895	3.315	5.065	5.489	<i>F</i>	13.27		0.04	4.94	0.15	0.05	0.949
	+	5.284	6.592	6.261	5.423	<i>P</i>	<0.005	3.92		<0.05			—
Blood	-	0.065	0.021	0.036	0.098	<i>F</i>	0.10		0.15	0.0	0.05	1.40	0.0014
	+	0.029	0.048	0.070	0.055	<i>P</i>		2.46					

of growth hormone (which has been postulated to be on muscle protein synthesis) develops fully. A second possibility is that asparagine has more than one function in protein synthesis: besides providing substrate for asparaginyl-tRNA formation, it might also promote the binding of mRNA, or even of aminoacyl-tRNA, to ribosomes. If growth-hormone-induced growth involves the production of at least some new mRNA in muscle, during an initial phase of 2–3 h, it is possible that raising the muscle asparagine concentration too early would favour retention of old mRNA, and actually make the translation of new mRNA more difficult. This twofold action of amino acids was proposed by Jefferson & Korner (1969) (see also Turner, 1972).

The various effects of tryptophan in these experiments deserve some comment. The blood asparagine concentration when both asparagine and tryptophan were injected was only half that with asparagine alone. This could have been a consequence of an increased secretion of insulin, which lowers the plasma amino acid concentration (Hernandez & Coulson, 1961). However, injection of tryptophan significantly raised both the asparagine and aspartate concentrations in brain, and it seems possible that the lowered blood asparagine concentration after tryptophan injection was a consequence of an increased uptake of asparagine into brain and a correspondingly high rate of hydrolysis to aspartate and NH_3 . The rise in brain aspartate concentration after asparagine injection (Table 2) is statistically highly significant, and suggests strongly that rapid conversion of asparagine into aspartate can occur. The possibility that the forebrain controls the blood asparagine concentration certainly merits investigation. The

increase of aspartate concentration in kidney after tryptophan injection 3 h after growth hormone cannot at present be explained.

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