Thyroxine: Effects on Amino Acid Incorporation into Protein in vivo

Abstract: *Treatment of rats with* L-*thyroxine increases the incorporation in vivo of radioactive amino acids into protein of liver, kidney, and heart, but not of spleen, testis, or brain. The distribution of the effect among the organs is the same as that observed in thyroxine stimulation of oxidative metabolism. Thus, stimulation of protein synthesis seems to be a physiological action of the thyroid hormone.*

The effects of thyroid hormones on protein biosynthesis have received considerable attention in recent years. In 1952, Du Toit (*1*) observed that liver slices from thyroidectomized rats showed low rates of amino acid incorporation into protein which could be raised toward normal by treatment of the animals with L-thyroxine (*1*). Further evidence of a reduced rate of protein biosynthesis in hypothyroidism and its return to normal after effective thyroid therapy has been obtained in intact man by Crispell, Parson, and Hollifield (*2*); in one euthyroid subject given triiodothyronine, however, protein synthesis appeared to be decreased. In 1959, Sokoloff and Kaufman (*3*) reported that prior administration of L-thyroxine to normal animals or its addition to the incubation medium directly in vitro stimulated the rate of amino acid incorporation into protein in cell-free rat-liver homogenates (*3*); in similar preparations from thyroidectomized rats the rate of amino acid incorporation was low (*4*). On the basis of these and related findings they suggested that the characteristic action of thyroxine on oxygen consumption might be secondary to the stimulation of energy-requiring processes such as protein biosynthesis (*3*). Subsequent studies by these workers and their associates (*4, 5*) further characterized the thyroxine stimulation of protein biosynthesis and localized it to the step in protein synthesis involving the transfer of soluble RNA-bound amino acid to microsomal protein. The effect of thyroxine added in vitro has now also been observed in slices of rabbit bone marrow by Necheles (*6*), in cell-free rat liver homogenates by Kamei, Michel, and Roche (*7*), and in slices of rat kidney by Hanson, Lindsay, and Barker (*8*). The effect of thyroxine treatment in vivo has been confirmed in cell-free rat liver preparations from thyroidectomized rats by Stein and Gross (*9*) and most recently by Tata and his associates (*10*).

In all but one of the studies cited, protein synthesis was assayed in vitro. In the one exception (*2*), the technique employed (*11*) was indirect and allowed inferences concerning the rate of protein synthesis only if certain assumptions, which might be hazardous to make in the case of hyperthyroidism, are valid. There has, therefore, been no satisfactory demonstration that the thyroxine stimulation of protein biosynthesis assayed in vitro represents a true physiological action of the hormone. In our study, protein biosynthesis in vivo has been examined directly by measurement of the rate of incorporation of radioactive amino acids into tissue proteins in the intact animal. The results indicate that the thyroxine stimulation of amino acid incorporation into protein observed in vitro also occurs in vivo in those organs which respond to thyroxine with increased metabolic rates.

Normal male Osborne-Mendel rats weighing between 65 and 115 g were paired for age and weight. One member of each pair received 100 μg of sodium L-thyroxine dissolved in 0.5 or 1.0 ml of 0.01*N* NaOH by intraperitoneal injection daily for 2 or 3 days; the control animal received equivalent volumes of the solvent alone. Both members of each pair were then treated identically through all subsequent procedures in accordance with paired experimental design. The incorporation of amino acid into protein was assayed simultaneously in paired animals on the day after the last injection. The animals were either fasted or allowed 20 percent glucose in the drinking water ad libitum for at least 18 hours immediately before the experiment.

Amino acid incorporation into protein was determined by measurement of the specific activity of various tissue proteins after the parenteral administration of L-leucine-1-C^{14}. In most experiments the L-leucine-1-C^{14} (specific activity, 5.7 mc/mmole) was dissolved in normal saline to a final concentration of 1.25 μc/ml and infused continuously by way of the tail vein at a constant rate of 0.05 ml/min by means of an infusion pump (Harvard Apparatus Co. model No. 600-910) for 30, 60, or 120 minutes. In a few experiments the L-leucine-1-C^{14} (specific activity, 6.8 mc/mmole) was administered by a single intraperitoneal injection of 2.5 μc of the amino acid in 1 ml of normal saline, and specific activity of the tissue proteins was determined 40 to 60 minutes after the injection. At the end of the infusion or the specified time, the animals were decapitated; the various organs were removed as rapidly as possible, blotted free of blood, and frozen in liquid nitrogen. A fraction of each organ was then homogenized in 6 percent trichloroacetic acid, and the precipitated protein was purified, plated, and assayed for specific activity (*3, 4*).

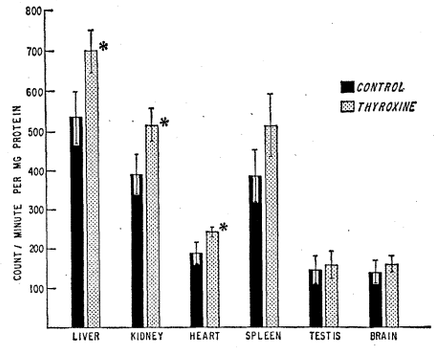

Fig. 1. The incorporation of L-leucine-1-C^{14} into protein in six organs of six paired thyroxine-treated and control rats infused intravenously with labeled amino acid for 120 minutes. The values represent means and standard errors. The asterisk indicates statistically significant difference ($p < 0.05$ as determined by method of paired comparison).

The results on the six organs examined simultaneously in six pairs of control and thyroxine-treated rats after 120 minutes of infusion of the radioactive amino acid are presented in Fig. 1. Thyroxine treatment significantly increased incorporation of L-leucine-1-C^{14} into protein in the liver, kidney, and heart. No significant effects were observed in the spleen, testis, and brain. Thyroxine also does not stimulate amino acid incorporation into protein in cell-free brain preparations from rats of this age (*12*). Although there is a difference between the mean values in the spleen, this difference is not statistically significant because of great individual variation in the specific activities of spleen protein. The total spleen weight also varied widely. The variations in both total weight and protein specific activity may reflect differences in agonal contraction and trapping of blood in the organ before its removal.

The thyroxine effects seemed unre-

Table. 1. Relationship of mode of amino acid administration to the effects of L-thyroxine on the incorporation of L-leucine-1-C^{14} into protein in various organs. The values are the mean of individual percentage differences between paired control and thyroxine-treated rats in numbers of paired experiments indicated in parentheses.

Duration of amino acid dose (min)	Thyroxine effects in various organs (%)					
	Liver	Kidney	Heart	Spleen	Testis	Brain
			Continuous intravenous infusion			
30	28* (7)	3 (1)	88 (1)	−80 (1)	−38 (1)	26 (1)
60	7 (14)	37 (5)	4 (7)	3 (4)	−3 (4)	−7 (4)
120	19* (13)	42† (6)	27†(11)	49 (6)	17 (6)	20 (6)
Total	16* (34)	37†(12)	21†(19)	20 (11)	5 (11)	10 (11)
			Single intraperitoneal injection			
	37† (7)	28 (3)	43 (3)	4 (3)	2 (3)	9 (3)

* $p < 0.01$; † $p < 0.05$ } determined by method of paired comparison.

lated to the duration of the infusion or the mode of administration of the labeled amino acid. Although in some cases there were insufficient numbers of experiments for adequate statistical analysis, there is evidence (Table 1) of stimulations of the incorporation of L-leucine-1-C^{14} into protein in liver, kidney, and heart after each of the periods of continuous intravenous infusion or the single intraperitoneal injection. On the other hand, there appear to be no consistent effects of thyroxine in the spleen, testis, or brain regardless of the duration or mode of administration. The thyroxine stimulations in liver were significant in both the fasted and glucose-fed animals but were more consistent in the latter, an observation previously made on the effects of thyroxine on other biosynthetic pathways (*13*) and attributed to the rapid depletion of liver glycogen in the fasted, thyrotoxic animal.

The results after the single intraperitoneal injection are at variance with those reported in which thyroxine treatment appeared to reduce incorporation of the labeled amino acid (*14*); however, in those studies the protein specific activity was assayed several hours after the injection of amino acid when the radioactivity of the precursor amino acid pool was negligible and protein breakdown rather than synthesis was the major determinant of protein specific activity.

Increased incorporation of the radioactive amino acid is not in itself conclusive evidence that thyroxine stimulates protein biosynthesis in vivo. Incorporation of the radioactive amino acid is dependent not only on protein synthesis but also on the history of the specific activity of the precursor amino acid pool. Measurements of the specific activity of the free leucine in the same tissues in which specific activity of protein was measured are currently in progress. There are, however, already a number of indications that the thyroxine stimulation of L-leucine-1-C^{14} incorporation into protein cannot be attributed to an effect on pool specific activity. The specific activity of the free-leucine pool in the tissue is dependent on the rate of delivery of the L-leucine-1-C^{14} relative to the size of the tissue leucine pool as well as to the rate of metabolic turnover of this pool. Circulatory effects would be most apparent early in the infusion procedure and would tend to disappear as the infusion continued and the specific activity of the tissue free-leucine pool approached equilibrium with that of the infused amino acid. However, in these studies the thyroxine effect appears to be unrelated to the duration of infusion or, if anything, more prominent with the prolonged infusions (Table 1). Also the thyroxine effects appear to be similar by two entirely different modes of administration. Evidence in the literature suggests that in thyrotoxicosis, free amino acid or leucine pools in the tissues are unchanged or increased (*15*), a change which would tend to diminish the incorporation of the labeled amino acid into protein. Finally, increased turnover of the amino acid pool, which almost certainly occurs in the hyperthyroid state, would result in continuous dilution of the administered labeled amino acid by unlabeled amino acid from endogenous sources and would, therefore, also tend to diminish the incorporation into protein of the labeled species.

Thus, our results strongly suggest that thyroxine stimulates protein biosynthesis in vivo as well as in vitro. Furthermore, the pattern of stimulation of amino acid incorporation in various organs is strikingly similar to the pattern of effects of thyroxine on oxygen consumption (*16*). Thyroxine appears to stimulate both amino acid incorporation and oxygen consumption in the liver, kidney, and heart but has no effect on either process in the spleen, testis, and brain (*16*). The effects on amino acid incorporation, therefore, parallel the effects on an accepted physiological action of the hormone, indicating that the action of thyroxine on protein biosynthesis, previously observed in vitro is not simply a laboratory curiosity but a true physiological action of the thyroid hormone. Furthermore, it has recently been shown that puromycin inhibition of protein synthesis (and, therefore, also of any thyroxine effect on protein synthesis) acutely reduces the metabolic rate in thyrotoxicosis to normal levels (*17*). This finding suggests that the effects of thyroxine on oxidative metabolism are secondary to its effects on protein synthesis.

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