IN VITRO STIMULATION OF THE HEXOSE MONOPHOSPHATE PATHWAY IN THYROID BY THYROID STIMULATING HORMONE

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The mechanism whereby thyroid stimulating hormone (TSH) increases thyroid function has not been established although several effects have been observed. The earliest in vitro effect previously reported was a small increase in oxygen consumption 10 minutes after TSH addition to thyroid slices (Freinkel 1957) while somewhat delayed stimulation of iodide trapping, organification, thyroid hormone release (Bakke and Lawrence 1956) and phospholipid synthesis (Morton and Schwartz 1953) have also been demonstrated.

In thyroid slices we have observed a ratio of greater than unity when $C^{14}O_2$ derived from glucose-1-C^{14} is compared to $C^{14}O_2$ obtained from glucose-6-C^{14} (Table 1). These results have been interpreted as evidence for the existence of the hexose monophosphate pathway in this tissue (Bloom and Stetten 1953). Similar results were also obtained by Dumont (1959). The existence of some of the enzymes necessary for this pathway in thyroid tissue has been noted previously (Schussler and Ingbar 1959). The present communication reports that TSH preferentially stimulates the oxidation of glucose-1-C^{14} to $C^{14}O_2$ with a much smaller increase in $C^{14}O_2$ derived from glucose-6-C^{14}.

Slices from calf thyroid were prepared, lightly blotted and weighed. Each slice was placed in a flask containing 2 ml. of Krebs-Ringer bicarbonate buffer (pH 7.4), 5 mg. of glucose and 0.5 μc of either glucose-1-C^{14} or

glucose-6-C^{14} (purchased from the National Bureau of Standards). All substances tested were dissolved in buffer. The flasks were gassed with 95% O_2 and 5% CO_2 and shaken at 37°C for 45 minutes. At the end of the incubation 1 ml. of hyamine base was placed in the center well and the reaction stopped by the addition of 0.2 ml. of 10 N H_2SO_4. Then the flasks were shaken for 60 minutes at room temperature to trap $C^{14}O_2$ in the hyamine. The hyamine containing $C^{14}O_2$ was counted in a liquid scintillation counter. Results are expressed as counts/min./gm. of tissue.

Table 1

Specificity of TSH Stimulation of $C^{14}O_2$ Production by Thyroid Slices

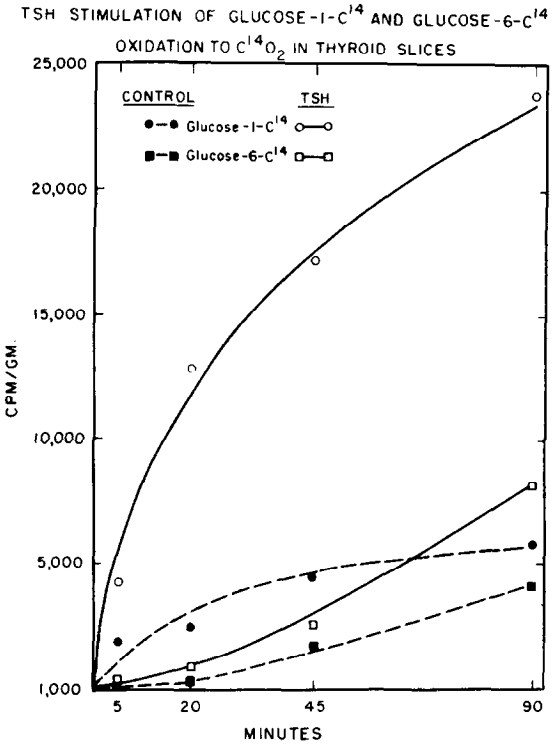
Drug	Amount per Flask	$C^{14}O_2$ (cpm/gm.) Derived From		Ratio $\frac{C^1}{C^6}$
		Glucose-1-C^{14}	Glucose-6-C^{14}	
Control		5,975	2,146	2.9
TSH*	1 unit	43,425	4,843	9.0
ACTH	1.5 units	5,450	1,090	5.0
Prolactin**	20 units	5,690	1,760	3.2
FSH**	1 mg.	9,975	2,345	4.3
Growth Hormone**	1 mg.	5,510	1,888	2.9

*Gift of Dr. Peter Condliffe, National Institutes of Health (5 units/mg.).
**Gift of Endocrine Study Section, National Institutes of Health.

One unit of TSH markedly stimulated the oxidation of glucose-1-C^{14} to $C^{14}O_2$ (Table 1). There was a lesser effect from 0.1 unit but none from 0.01 unit. Figure 1 illustrates that this effect of TSH is manifest within five minutes after the start of the incubation. Since this effect is earlier than all those previously reported in vitro, it suggests that the primary action of TSH might be on glucose metabolism by the thyroid. No consistant increase in glucose uptake by thyroid slices was noted during a 90 minute incubation with TSH.

This effect of TSH was obtained with both commercial TSH (Thytropar, Armour) and a more purified preparation.* There was no stimulation of the hexose monophosphate pathway by adrenocorticotropin (ACTH), prolactin, or growth hormone although there was a small effect from follicle stimulating hormone (FSH) (Table 1). This effect of FSH was probably due to TSH contamination (Bates 1959). TSH did not stimulate this pathway in liver

Fig. 1



slices. This evidence demonstrates that the TSH effect on thyroid slices appears to be specific despite the fact that relatively large amounts of the hormone must be used. However the amount used is equivalent to that necessary to produce other in vitro effects (Morton and Schwartz 1953).

Recently the importance of the hexose monophosphate pathway as a source of TPNH for synthetic reactions has been stressed (Siperstein 1958). It is possible that the increased generation of TPNH could account for the other in vitro effects which have been ascribed to TSH.

*Gift of Dr. Peter Condliffe, National Institutes of Health (5 units/mg.)

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