STEREOSPECIFIC SUGAR TRANSPORT CAUSED BY THYROID STIMULATING HORMONE AND ADENOSINE 3',5'-MONOPHOSPHATE IN THE THYROID GLAND AND OTHER TISSUES

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Thyroid stimulating hormone (TSH) has been shown to increase glucose oxidation, α-aminoisobutyric acid uptake, and incorporation of amino acids into protein in thyroid tissue _in vitro_ (Field _et al._, 1959; Debons and Pittman, 1962; Raghupathy _et al._, 1963). However, no substantial evidence has been obtained to suggest that TSH enhances the rate of entry of sugars into the cells of the thyroid tissue from the medium. The present experiments had three purposes: (1) to determine whether TSH facilitates the penetration of d-xylose into the thyroid gland, (2) if (1) is so, to determine whether the TSH effect on the penetration of pentose isomers into the thyroid gland has the same stereospecificity as the insulin effect in the muscle, and (3) to test whether pituitary hormones alter the sugar transport system in their target glands by stimulating the formation of adenosine 3',5'-monophosphate (cyclic AMP).

Methods

Thyroid glands were obtained immediately after calves were sacrificed at a slaughterhouse and were brought to the laboratory chilled on ice. Thyroid slices weighing 150 to 300 mg were prepared by hand with a razor blade. For each experimental flask, a control flask was set up; experimental and control flasks contained adjoining slices of thyroid. Adrenal preparations were obtained from exsanguinated white male rabbits; after being stripped of the thin fibrous capsule, adrenals weighing 120 to 200 mg were bisected for incubation. The tissues were incubated at 37°C with shaking (under 100 per cent O_2) in 5 ml of Krebs-Ringer phosphate containing pentose (4 mg/ml) for 90 minutes. One of each pair of flasks also contained TSH (1 M.S.E./ml) or cyclic AMP (1.5×10^{-3}M) dissolved in the buffer. The extracellular space was determined with inulin in a separate incubation and with C^{14}-sucrose in the presence of pentose. The TSH preparation used in these experiments was commercial material (Thyrotropes Hormon, Schering). Two preparations of cyclic AMP were employed, which were generously supplied by Takeda Chemical Industries, Ltd. and by Kowa Chemical Laboratory. Pentoses were determined by the p-bromoaniline method of Roe and Rice (1948) with corresponding standards, and inulin by the Roe ketose method (1949). C^{14}-sucrose was assayed in a gas flow counter. The water volume (W.V.) of tissues incubated under these conditions was measured by drying the material to a constant weight at 100°C, the value being expressed as a per cent of the wet weight of the tissues. The distribution of a substrate in whole tissue (space) is expressed by the following equation:

$$\text{Space (ml/100 gm)} = \frac{\text{content in wet tissue (mg/gm)}}{\text{medium concentration (mg/ml)}} \times 100 \qquad (1)$$

The intracellular distribution of pentose is then calculated as follows:

$$\text{Intracellular Distribution} = \frac{\text{P.S.} - \text{E.S.}}{\text{W.V.} - \text{E.S.}} \times 100 \qquad (2)$$

in which P.S. is the pentose space and E.S. is the extracellular space.

Results and Discussion

Tables 1 to 3 summarize the results in separate experiments. Each value in these tables is the average of results from 5 to 7 observations.

The data in Table 1 show that addition of TSH *in vitro* produced a significant increase in the d-xylose and l-arabinose spaces in bovine thyroid slices without causing any increase in the l-xylose or d-arabinose spaces. Calculated from equation (2), the intracellular distribution of d-xylose is increased about twofold by TSH, assuming inulin or sucrose to be a measure of the extracellular space. These responses of thyroid tissue to TSH are similar to those of diaphragmatic muscle to insulin except that in muscle preparations the l-xylose and d-arabinose spaces (especially the latter) are to a certain degree affected by insulin addition (Carlin and Hechter, 1961; Tarui and Nonaka, 1963). The effect of TSH on sugar transport seems to be organ specific, since the hormone had no effect upon the d-xylose space of bovine testis slices and bisected rabbit adrenals.

Table 1

Effect of TSH on the distribution of various sugars in bovine thyroid slices

	Control	With TSH (1 M.S.E./ml)	Net effect*	P value
d-xylose space	53.3	59.6	6.3 $\pm$ 2.4	$<.001$
l-xylose space	55.7	54.8	-0.9 $\pm$ 0.8	
d-arabinose space	55.8	54.5	-1.3 $\pm$ 2.1	
l-arabinose space	54.4	60.2	5.8 $\pm$ 1.4	$<.005$
inulin space	45.3	44.5	-0.8 $\pm$ 0.7	
C^{14}-sucrose space	48.4	48.9	0.5 $\pm$ 0.9	
water volume	81.3	80.9	-0.4 $\pm$ 0.9	

* Average $\pm$ standard deviation of difference between TSH- or AMP-treated materials and the corresponding controls (Table 1, 2 and 3).

Table 2

Effect of cyclic AMP on the distribution of various sugars in bovine thyroid slices

	Control	With AMP (1.5×10^{-3}M)	Net effect	P value
d-xylose space	53.7	57.7	4.0 $\pm$ 1.5	$<.01$
l-xylose space	53.5	51.8	-1.7 $\pm$ 1.1	$<.05$
d-arabinose space	54.5	52.5	-2.0 $\pm$ 1.7	
l-arabinose space	51.3	55.6	4.3 $\pm$ 1.4	$<.01$
inulin space	44.4	43.6	-0.8 $\pm$ 0.7	
C^{14}-sucrose space	50.3	49.8	-0.5 $\pm$ 0.5	
water volume	82.2	82.1	-0.1 $\pm$ 0.4	

Table 3

Effect of cyclic AMP on the distribution of various sugars in bisected rabbit adrenals

	Control	With AMP (1.5×10^{-3}M)	Net effect	P value
d-xylose space	24.9	29.8	4.9 ± 1.3	$<.005$
l-xylose space	27.1	25.8	-1.3 ± 1.7	
d-arabinose space	26.3	24.7	-1.6 ± 0.9	$<.02$
l-arabinose space	25.4	30.9	5.5 ± 2.4	$<.01$
C^{14}-sucrose space	21.6	21.5	-0.1 ± 0.5	
water volume	70.9	70.5	-0.4 ± 0.4	

It has not been determined whether TSH exerts its effect through cyclic AMP. As shown in Table 2, the stereospecific effect on sugar transport was reproduced when cyclic AMP was added to the medium instead of TSH. No effect of 5'-AMP was observed. It is suggested that TSH facilitates sugar transport in the thyroid gland by stimulating the formation of cyclic AMP.

The effect of cyclic AMP on sugar transport is not organ specific. This is clearly seen in Table 3, indicating that the penetration of pentose isomers into bisected rabbit adrenals was increased by cyclic AMP in the same manner as in bovine thyroid slices. Adrenocorticotropic hormone (ACTH) has been shown by Haynes (1958) to cause an accumulation of cyclic AMP in slices from adrenal cortex. Eichhorn et al. (1960) suggested that ACTH action involves the regulation of sugar permeability. It is quite possible that ACTH alters the sugar transport system in adrenal cortex by causing the accumulation of cyclic AMP.

No attempt has been made in these studies to determine the maximal effective dose of cyclic AMP. However, since cyclic AMP has a poor ability to enter into cells, exposure of the tissues to higher levels of this material would probably cause a more marked increase in the penetration of pentoses.

Summary

Addition of a TSH preparation to the incubation medium promoted the penetration of d-xylose and l-arabinose into bovine thyroid slices without causing any increase in the spaces of the corresponding optical isomers. These responses could be reproduced when cyclic AMP was added to the medium instead of TSH. When bisected rabbit adrenals were incubated in the presence of cyclic AMP, there was a stereospecific effect on sugar transport of the same type as observed in bovine thyroid.

References

Carlin, H., and Hechter, O., J. Gen. Physiol. 45, 309 (1961).

Debons, A. F., and Pittman, J. A., Endocrinology 70, 937 (1962).

Eichhorn, J., Halkerston, I. D. K., Feinstein, M., and Hechter, O., Proc. Soc. Exp. Biol. Med. 103, 515 (1960).

Field, J. B., Pastan, I., Johnson, P., and Herring, B., Biochem. Biophys. Res. Commun. 1, 284 (1959).

Haynes, R. C. Jr., J. Biol. Chem. 233, 1220 (1958).

Raghupathy, E., Tong, W., and Chaikoff, I. L., Endocrinology 72, 620 (1963).

Roe, J. H., and Rice, E. W., J. Biol. Chem. 173, 507 (1948).

Roe, J. H., Epstein, J. H., and Goldstein, N. P., J. Biol. Chem. 178, 839 (1949).

Tarui, S., and Nonaka, K., Endocrinol. Japon., in press (1963).