STIMULATION OF PITUITARY GLUCOSE OXIDATION BY THYROTROPIN-RELEASING HORMONE

J. A. Pittman, Jr., E. Dubovsky, and R. J. Beschi

Endocrinology and Metabolism Division, Department of Medicine, University of Alabama in Birmingham, and Nuclear Medicine Service, Veterans Administration Hospital, Birmingham, Alabama 35233

Received August 5, 1970

SUMMARY: Pituitary halves from normal or thyroidectomized rats were incubated in media containing glucose-1- or glucose-6-^{14}C and the $^{14}CO_2$ produced in 45 min was measured. Synthetic thyrotropin-releasing hormone (TRH) stimulated oxidation of glucose-6-^{14}C to $^{14}CO_2$ but not C-1 labeled glucose. The effect was most consistent and prominent in pituitaries from thyroidectomized rats and was blocked by the addition of thyroxine to the medium. TRH exerted no comparable stimulatory action on liver, brain, kidney, or muscle under comparable conditions.

Thyrotropin-releasing hormone (TRH) has recently been isolated, chemically characterized, and synthesized (1, 2). Small quantities of TRH are capable of releasing TSH (thyroid stimulating hormone, or thyrotropin) from the anterior pituitary of mammals, both *in vivo* and *in vitro* (3). The mechanisms by which this release occurs, or which accompany release, are not known and have thus far received little attention. The data in the present communication demonstrate that TRH can effect an increase in glucose oxidation by rat pituitaries *in vitro*.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 180 to 350 g body weight, were decapitated and the pituitary glands removed within 35 to 50 seconds, placed in Krebs-Ringer bicarbonate buffer, pH 7.4 at 0°C, and hemisected about 5 minutes later after removal of the neurohypophysis. The halves were placed in glass test tubes (7.0 x 1.5 cm) containing 0.22 ml of the

medium kept in iced trays. The tubes were gassed for 20 seconds with 95% O_2-5%CO_2 and capped with rubber diaphragms after insertion of pedunculated glass center wells. The glucose concentration in all experiments was 72 mg/100 ml, and the radioactivity was 0.5 μCi per vessel. One test tube containing a hemipituitary served as the experimental, while a second containing the other half of the same gland was the control.

The tubes were incubated in a Dubnoff shaker at 37°C for 45 minutes. After this period, the reaction was stopped by addition of 0.1 ml of 1N sulfuric acid to the medium via needle through the rubber diaphragm. One ml of hydroxide of hyamine ("10X", Packard Co., DesPlaines, Ill.) was added to the center well in the same manner, and the tubes were then shaken for an additional 60 minutes at room temperature. The hyamine was then transferred to counting vials with 15 ml of 0.4% PPO (2,5-diphenyloxazole) and 5% dimethyl POPOP (1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene) and counted at 72-74% efficiency. The results were corrected for quenching and are expressed here as dpm/mg protein as determined by the Lowry method (4).

Most experiments were carried out on pituitaries from rats which had been rendered hypothyroid by surgical thyroidectomy done 3 weeks prior to killing the animals.

In one experiment the capacity of TSH to stimulate oxidation of glucose-1-^{14}C by rat thyroid gland halves, approximately 5-6 mg each, was tested in a procedure similar to the one outlined above.

The TRH was synthetic material kindly supplied by M. S. Anderson, and W. F. White, Abbott Pharmaceutical Co., North Chicago, Ill., Lot No. 3252-104. The TSH was from the Division of Biological Standards, National Institute for Medical Research, Mill Hill, London, and was bovine with a specific activity of 0.074 I.U./mg. The L-thyroxine, sodium salt, pentahydrate, was purchased from the Sigma Chemical Co., St. Louis, Missouri. The D-glucose-6-^{14}C (4.74 mCi/mM) and D-glucose-1-^{14}C (5.63 mCi/mM) were purchased from the New England Nuclear Co., Boston, Mass.

Table 1

Stimulation of Glucose-6-^{14}C Oxidation in Rat Pituitary

Number of Pairs and State of Donor	$C^{14}O_2$ DPM/mg protein ± SEM		TRH nanograms/ml	Exper/Contr X 100
	Control	Experimental		
18 (Tx)	2510 ± 140	3440 ± 150	500	137 ***
5 (Tx)	2580 ± 160	3190 ± 163	100	123 **
5 (Tx)	2890 ± 300	3620 ± 230	50	125 *
4 (Tx)	2960 ± 120	3940 ± 270	5	133 *
26 (N)	4140 ± 250	5130 ± 330	500	123 ***
5 (N)	3220 ± 200	3850 ± 300	166.5	119 N.S.
5 (N)	3560 ± 303	3850 ± 304	55.5	108 N.S.

N = normal rats; Tx = thyroidectomized rats
*** p less than 0.001; ** p less than 0.01; * p less than 0.02
N.S. = No significant difference between control and experimental

RESULTS AND DISCUSSION

Table 1 shows the increase in production of labeled CO_2 from glucose-6-^{14}C brought about by addition of TRH to the medium in concentrations of 5 to 500 nanog/ml. The effect was most consistently and prominently seen in pituitaries obtained from hypothyroid rats, those from normal rats often showing no statistically significant response. The data in Table 2 show that the effect was not seen when glucose-1-^{14}C was substituted for C-6 labeled glucose. That this was not due to some error in the system is attested by the clear response to TSH seen in the rat thyroid lobes using the same reagents. TSH is known to cause preferential oxidation of glucose-1-^{14}C by thyroid tissue (5). The tissue specificity of the response to TRH is shown by the data in Table 3. Liver and brain from thyroidectomized animals and liver, brain, kidney and muscle from normal animals did not respond. The addition of thyroxine to the medium blocked the stimulatory action of TRH, as shown in Table 4.

Table 2

Glucose-1-^{14}C Oxidation in Rat Pituitary and Thyroid

State of Donor Rat	Number of Pairs	$C^{14}O_2$ DPM/mg Protein ± SEM		TRH or TSH per ml	Exper/Control X 100
		Control	Experimental		
N	7	11620 ± 530	11580 ± 340	TRH 500 nanog	99 N.S.
Tx	7	6680 ± 380	6850 ± 750	500 nanog	102 N.S.
Tx	2	5420 ± 180	5420 ± 65	5 μg	100 N.S.
Thyroid Halves	3	5270 ± 202	7950 ± 245	TSH 10 mU	151 **

N = normal rats; Tx = thyroidectomized rats
** p less than 0.01; N.S. = no significant change

Table 3

Stimulation of Glucose-6-^{14}C Oxidation in Tissues of Normal and Thyroidectomized Rats

Tissue	Number of Samples and State of Donor	$C^{14}O_2$ DPM/mg protein ± SEM		TRH nanograms/ml	Exper/Contr X 100
		Control	Experimental		
Pituitary	52 (N)	4140 ± 250	5130 ± 330	500	123 ***
Kidney	12 (N)	12820 ± 770	13070 ± 840	500	102 N.S.
Liver	8 (N)	2680 ± 230	2040 ± 140	500	76 N.S.
Brain	12 (N)	9730 ± 940	9970 ± 1080	500	102 N.S.
Muscle	6 (N)	1430 ± 160	1070 ± 250	500	75 N.S.
Pituitary	36 (Tx)	2510 ± 140	3440 ± 150	500	137 ***
Liver	8 (Tx)	1430 ± 100	1270 ± 200	500	89 N.S.
Brain	2 (Tx)	9830	8510	500	87 N.S.

N = normal rats; Tx = thyroidectomized rats
*** p less than 0.001; N.S. = no significant difference between control & experimental

Table 4

Influence of TRH and T_4 on Glucose-6-Oxidation on Pituitary of Thyroidectomized Rats

No. of Pairs	$C^{14}O_2$ DPM/mg protein ± SEM		Material added/ml	Exper/Control
	Control	Experimental		
5	2010 ± 60	2710 ± 170	TRH 500 nanog	134 **
5	2400 ± 120	2150 ± 40	T_4 3.22 x 10^{-5}M	89 N.S.
5	2460 ± 220	2500 ± 90	TRH 500 nanog T_4 3.22 x 10^{-5}M	101 N.S.

** = p less than 0.01; N.S. = no significant difference between control and experimental; T_4 = L-thyroxine

Other agents have been reported to stimulate pituitary glucose oxidation. Barondes et al showed stimulation of glucose-1-^{14}C oxidation by epinephrine, norepinephrine, and serotonin, but no stimulation was observed with glucose-6-^{14}C (6). They ascribed the effects of these amines to their metabolism to alcohols with subsequent enhanced formation of $NADP^+$ from NADPH (7). Krass et al observed stimulation by vasopressin, oxytoxin, and epinephrine, the effect of the last being marked (8). However, this stimulation of glucose oxidation occurred at higher concentrations of the hormones than were required for TSH release into the medium, and the two effects could also be dissociated by incubating the tissue at low temperature (8).

The concentrations of TRH used in these experiments, 5 to 500 nanog/ml (or 1.4 x 10^{-8} to 1.4 x 10^{-6}M), while small on an absolute scale, are much larger than the doses capable of stimulating TSH release in vitro (3.7 x 10^{-11}M) (3). We used thyroidectomized rats on the assumption that the pituitaries from such animals would contain a larger proportion of thyrotroph cells than glands from normal rats, and the results indicate that the former do give a greater response, starting from a lower basal rate of oxidation. These results, together with the blockade of the TRH effect produced by

thyroxine, suggest that the effect observed may be related to the physiologic action of the hormone.

The preferential stimulation of oxidation of the sixth carbon of glucose and absence of any effect on the first carbon is unusual. A similar effect has been reported for growth hormone's action on adipose tissue (9). Preliminary observations suggest that dibutyryl cyclic adenosine monophosphate (dibutyryl cAMP) causes a pattern of stimulation similar to that reported here for TRH. If this is so, the TRH effect on glucose oxidation may be a relatively nonspecific one resulting from enhanced formation of cAMP in pituitary. Such enhancement has recently been reported to result from addition of hypothalamic extracts to incubated pituitaries (10).

Further experiments are in progress to evaluate the effects of lower doses of TRH in our system and to examine the mechanisms by which the effect occurs.

ACKNOWLEDGEMENT

We are grateful to Kinnis Kelly for his excellent technical assistance in carrying out these studies.

REFERENCES

1. Folkers, K., Enzmann, F., Boler, J., Bowers, C. Y., and Schally, A. V., Biochem. Biophys. Res. Comm. 37:123, 1969.
2. Burgus, R., Dunn, T. F., Desiderio, D., and Guillemin, R., C. R. Acad. Sc. (Paris) 269:1870, 1969.
3. Mittler, J. C., Redding, T. W., and Schally, A. V., Proc. Soc. Exp. Biol. Med. 130:406, 1969.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. 193:265, 1951.
5. Field, J. B., Metabolism 17:226, 1968.
6. Barondes, S. H., Johnson, P., and Field, J. B., Endocrinology 69:809, 1961.
7. Barondes, S. H., and Field, J. B., Biochim. Biophys. Acta 73:324, 1963.
8. Krass, M. E., LaBella, F. S., and Vivian, S. R., Endocrinology 82:1183, 1968.
9. Winegrad, A. I., Shaw, W. N., Lukens, F. D. W., Stadie, W. C., and Renold, A. E., J. Biol. Chem. 234:1922, 1959.
10. Steiner, A. L., Peake, G. T., Utiger, R. D., Karl, I. E., and Kipnis, D. M., Endocrinology 86:1354, 1970.