RAT DIAPHRAGM METABOLISM IN THE PRESENCE OF THYROACTIVE HORMONES, PRECURSORS AND GOITROGENS

LEON L. GERSHBEIN

Northwest Institute for Medical Research, 5656 West Addison Street, Chicago

ABSTRACT.—Mean changes in oxygen and glucose uptake and glycogen content of the isolated rat diaphragm incubated in phosphate-saline medium containing 120 mg % of glucose have been evaluated in the presence of 3-iodotyrosine (1.00 mg), 3,5-diiodotyrosine (1.00 mg), D-thyroxine Na • $5H_2O$ (0.25 mg), L-thyroxine Na • $5H_2O$ (0.25 and 2.50 mg), 3,5,3'-triiodothyronine Na (0.25 mg), bovine thyroglobulin (1.00 mg), thiourea (1.00 mg), 6-propyl-2-thiouracil (0.25 mg) and DL-thyronine (0.10 and 1.00 mg) and were found to be significant only for the last agent and at the higher level. In this case, the Q_{O_2} and glycogen content were markedly depressed. Possibly, a borderline significance can be attached to the decrease in mean glucose content observed with TSH (1.67 IU).

Reisser (1947) interpreted equivocal data as perhaps pointing to an increase in rat diaphragm glycogen synthesis with thyroxine (0.1-0.5 mg) or rat thyroid extract in a medium containing 500 mg % of glucose. This paper presents a pharmacological study undertaken on the isolated rat diaphragm to elucidate a possible role of thyroxine and triiodothyronine and precursors in muscle glucose utilization and glycogen turnover.

MATERIALS AND METHODS

Except for the bovine thyroglobulin and TSH obtained from Sigma Chemical Company, the source of the test agents was Nutritional Biochemicals Corporation. The TSH contained 1 U.S.P. unit (or 1 IU) per mg and was reported to be almost free of STH, gonadotrophins, ACTH and posterior pituitary activities. The phosphate-saline medium of Stadie and Zapp (1947) was employed in the incubation of diaphragms, the glucose content being 120 mg %. It was prepared double strength. The test agents were dissolved in physiological saline and the pH brought to 7.0 with sodium hydroxide in the case of the iodo-tyrosines and DL-thyronine. Saline or a solution of saline containing the specified weight of test agent in 1.0 ml was added to an equal volume of double strength Stadie phosphate-glucose medium per Warburg vessel. These comprised the control and treatment flasks, respectively.

Male rats purchased from the Holtzman Rat Company, Madison, Wisconsin, and weighing 130-165 gm at the time of experimentation, were starved for 24 hr in order to deplete diaphragm glycogen. They were sacrificed by swift decapitation, incised and the hemidiaphragms rapidly removed. Care was taken to avoid trauma and cutting of the vena cava. The hemidiaphragms were chilled in saline, trimmed, blotted between filter paper, weighed and introduced into

the Warburg flasks. One hemidiaphragm was incubated with the phosphate-saline control and the other muscle portion, with the treatment mixture or medium plus test agent. Controls which contained the media without tissue were also included. The flasks were connected to the manometers, the system gassed with oxygen for 10 min. and incubated in the bath at 37.5° C for 1 hr. The hemidiaphragms were then removed, rinsed with saline, blotted and immediately digested with 30% KOH. The glycogen which was precipitated in the presence of sodium sulfate on addition of ethanol according to the Walaas and Walaas (1950) modification of the Good, Kramer and Somogyi procedure (1933) was determined by Dreywood's anthrone reagent (Morris, 1948). Glucose in the incubated mixtures was analyzed following dilution 1:5 with water and deproteinization (Nelson, 1944; Somogyi, 1945). Six rats were employed per Warburg run and involving two test agents.

Results and Discussion

Mean changes in rat diaphragm Qo_2 glucose uptake and glycogen content in the presence of mono-and diiodotyrosines (each at a level of 1.00 mg), D-thyroxine (0.25 mg), L-thyroxine (0.25 and 2.50 mg), 3,5,3′-triiodothyronine (0.25 mg), DL-thyronine (0.10 and 1.00 mg), bovine thyroglobulin (1.00 mg), TSH (1.67 IU), thiourea (1.00 mg) and propylthiouracil (0.25 mg) together with the pertinent Fisher t values appear in Table 1. The levels of thyroxine and triiodothyronine are in terms of the sodium salts as specified. The contents of the flask containing 2.5 mg of the thyroxine salt occurred as a fine cloudy suspension. A statistical method which may be applied only to bulk data and where an experimental design is not followed, was employed to eliminate any possible questionable data. Individual differences were excluded which exceeded $R \pm 2.5\ R$, R being the average range (Grant, 1952).

At the given concentrations, none of the test agents elicited any statistically significant effect on the mean oxygen and glucose uptakes or the glycogen content of the isolated rat diaphragm except for DL-thyronine in amount of 1.00 mg or 3.7 μM. With the latter, Qo_2 and glycogen were both definitely depressed, but the mean decrease in glucose uptake was not marked; the corresponding results were equivocal with 0.10 mg of the thyronine as well as with the L-thyroxine salt at the comparably high level.

Attention is called to the case of TSH (1.67 IU) which caused a depression in mean glucose content of low or only borderline significance (t, 2.09; $P = 0.05$), findings which might be in contrast to those observed with isolated thyroid. Thus, Field and coworkers (1960) reported that TSH produced an increase in glucose uptake by thyroid slices.

Acknowledgement

The author is indebted to Messrs. Frank Bugaj and Alan Miller for technical assistance.

Literature Cited

Field, J. B., I. Pastan, P. Johnson, and B. Herring. 1960. Stimulation in vitro of pathways of glucose oxidation in thyroid by thyroid-stimulating hormone. J. Biol. Chem., 235: 1863-1866.

TABLE 1.—Mean Changes in Oxygen Uptake and Glucose and Glycogen Contents of Rat Diaphragms Incubated with Iodoamino Acids, Thyronine, TSH and Goitrogens[a,b].

Agent (mg)	Mean Q_{O_2} Change		Mean Glucose Difference[c]		Mean Glycogen Difference	
	μl/mg wet tissue/hr	t	μg/mg wet tissue/hr.	t	μg/mg wet tissue/hr	t
3-Iodo-L-tyrosine (1.00)	—0.04 ± 0.084 (19)	0.46	0.19 ± 0.643 (17)	0.29	0.01 ± 0.057 (17)	0.21
3, 5-Diiodo-L-tyrosine (1.00)	0.01 ± 0.048 (10)	0.18	0.62 ± 0.485 (8)	1.27	0.06 ± 0.047 (11)	0.86
DL-Thyronine (0.10)	0.06 ± 0.048 (14)	1.26	0.50 ± 0.391 (13)	1.27	0.06 ± 0.037 (13)	1.60
DL-Thyronine (1.00)	0.17 ± 0.045 (17)	3.76**	0.25 ± 0.333 (15)	0.76	0.25 ± 0.062 (16)	4.08**
D-Thyroxine (Na)·$5H_2O$ (0.25)	0.12 ± 0.063 (12)	1.83	0.28 ± 0.354 (11)	0.80	0.02 ± 0.067 (12)	0.32
L-Thyroxine (Na)·$5H_2O$ (0.25)	—0.01 ± 0.047 (22)	0.07	0.13 ± 0.431 (10)	0.31	-0.04 ± 0.035 (23)	1.06
L-Thyroxine (Na)·$5H_2O$ (2.50)	—0.03 ± 0.048 (12)	0.67	—0.88 ± 0.793 (10)	1.11	—0.11 ± 0.094 (13)	1.21
L-3, 5, 3'-Triiodothyronine (Na) (0.25)	0.01 ± 0.065 (10)	0.18	0.62 ± 0.475 (9)	1.31	—0.01 ± 0.032 (11)	0.30
Bovine thyroglobulin (1.00)	0.03 ± 0.051 (9)	0.60	—0.48 ± 0.301 (11)	1.59	0.05 ± 0.090 (10)	0.58
TSH (1.67 IU)	—0.01 ± 0.032 (19)	0.44	0.72 ± 0.344 (21)	2.09*	—0.01 ± 0.047 (21)	0.30
Thiourea (1.00)	- 0.07 ± 0.049 (16)	1.36	1.34 ± 0.772 (11)	1.74	—0.07 ± 0.076 (15)	0.86
6-Propyl-2-thiouracil (0.25)	0.04 ± 0.068 (13)	0.62	—0.04 ± 0.372 (11)	0.11	0.09 ± 0.050 (15)	1.80

a. The means (± S.E.) are deduced from the specific number of paired hemidiaphragms given in the parentheses.
b. A positive mean difference indicates a decrease in response in the presence of the test agent.
c. The extent of glucose utilization was based on the final concentration of the respective media incubated without diaphragm.
** $P < 0.01$.
* $P = 0.05$.

GOOD, C. A., H. KRAMER, and M. SOMOGYI. 1933. The determination of glycogen. J. Biol. Chem., 100: 485-491.

GRANT, E. L. 1952. Statistical quality control. Maple Press Co. New York, xvi + 557 pp.

MORRIS, D. L. 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science, 107: 254-255.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153: 375-380.

REISSER, O. 1947. Über Glykogensynthese im überlebenden Rattenzwerchfell und ihre Beeinflussung in vitro durch Hormone und Vitamine. Biochim. Biophys. Acta, 1: 208-233.

SOMOGYI, M. 1945. A new reagent for the determination of sugars. J. Biol. Chem., 160: 61-68.

STADIE, W. C., and J. A. ZAPP, JR. The effect of insulin upon the synthesis of glycogen by rat diaphragm in vitro. J. Biol. Chem., 170: 55-65.

WALAAS, O., and E. WALAAS. 1950. Effect of epinephrine on rat diaphragm. J. Biol. Chem., 187: 769-776.

Manuscript received August 11, 1964.