Effect of Synthetic Thyrotropin-Releasing Hormone on Glucose Oxidation and Phospholipogenesis in Porcine Anterior Pituitary Slices in the Presence or Absence of Thyroid Hormone (37058)

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Since thyrotropin-releasing hormone (TRH) was isolated and then synthesized (1, 2), a body of evidence has accumulated that TRH can stimulate pituitary secretion of thyrotropin (TSH) *in vivo* and *in vitro* (3, 4). Theoretically, alterations in the patterns of intermediary metabolism may be produced in the anterior pituitary in response to TRH stimulation, but the sequence of such event has not received much attention. Pittman, *et al.* (5) and Yamamoto *et al.* (6) have recently reported that synthetic TRH stimulated the oxidation of ^{14}C labeled glucose in rat and porcine anterior pituitaries. However, this TRH effect varied greatly from species to species, depending on which type of labeled glucose was used. Thus we have studied TRH effect on the oxidation of ^{14}C-6-glucose or of ^{14}C-1-glucose by porcine anterior pituitary. Furthermore, a possible role of TRH in pituitary phospholipogenesis was studied. Finally, we have studied the effect of thyroid hormone and/or actinomycin D on an increase of pituitary glucose oxidation and phospholipogenesis produced by TRH.

Materials and Methods. Synthetic TRH was kindly donated by the Abbott Laboratories, Japan. Dibutyryl cyclic 3′,5′-monophosphate (DBcAMP) was purchased from Boehringer Mannheim Japan Co. TRH was dissolved in saline buffered with 0.01 *M* sodium phosphate (pH 7.4) (PBS) just before use. The sodium salt of thyroxine or triiodothyronine was dissolved in 0.1 *N* sodium hydroxide, diluted with PBS and added to preincubation and incubation media.

Porcine pituitary glands were obtained from an abattoir. Three to four porcine pituitary glands were usually used for one experiment. After gentle separation of anterior and posterior pituitaries, slices 0.5 mm thick and weighing 60–80 mg were made with a Stadie-Riggs microtome. Each slice horizontally cut was further divided into two; one was used as a control and other as an experimental. Slices were lightly blotted on filter paper and weighed. For estimation of the rate of glucose oxidation, slices were incubated in medium containing ^{14}C labeled glucose, and the evolved $^{14}CO_2$ was collected in Hyamine base and counted in a liquid scintillation counter, as previously described (6). For the phospholipid synthesis, each slice was placed in a 15 ml Erlenmeyer flask containing 2 ml of Krebs–Ringer bicarbonate buffer with 5 μCi of orthophosphate ^{32}P/ml and 0.5 mg of glucose/ml, in addition to desired concentration of test substances. The flasks were gassed with 95% O_2–5% CO_2 and incubated in a Dubnoff metabolic shaker at 37° for 1 hr. At the end of incubation, slices were removed and washed successively with chilled 0.9% (w/v) NaCl and 2.11% (w/v) KH_2PO_4. Phospholipids were isolated by the method of Kögl and Van Deenen (7). Four-milliliter aliquots of the phospholipid extract were added to 10 ml of toluene containing 0.4% diphenyloxazole and 0.005% 1, 4-bis-2(5-phenyloxazole)-benzene in counting vials and counted in a liquid scintillation counter. The values of $^{14}CO_2$ produced and ^{32}P incorporated phospholipids in control flasks were usually 50–150 cpm/mg/hr and those in experiments were always expressed as percentage of control. Statistical analyses were carried out by the Student's t test. A p value < 0.05 was considered significant.

TABLE I. Effect of TRH on Pituitary Glucose Oxidation *in Vitro*.[a]

Expt	Groups	Addition to incubation medium (μg/ml)	^{14}C labeled glucose	No. of determinations	$^{14}CO_2$ produced (% of control)	*p* value from control
1.	A	PBS	C-6	5	100.0 ± 6.4[b]	
	B	TRH, 0.5	C-6	5	149.1 ± 11.6	<.01
	C	TRH, 5.0	C-6	5	166.3 ± 11.1	<.01
	D	TRH, 50.0	C-6	5	100.9 ± 7.7	
2.	A	PBS	C-1	5	100.0 ± 4.9	
	B	TRH, 0.5	C-1	5	116.1 ± 5.8	<.05
	C	PBS	C-6	5	100.0 ± 4.7	
	D	TRH, 0.5	C-6	5	147.5 ± 20.3	<.05
	E	TRH, 0.1	C-6	5	176.4 ± 20.4	<.05
3.	A	PBS	C-U	4	100.0 ± 7.4	
	B	TRH, 5.0	C-U	4	137.4 ± 10.6	<.05
	C	T_4, 1.0 + TRH, 5.0	C-U	4	92.8 ± 10.2	
	D	Act D, 10 + T_4, 1.0 + TRH, 5.0	C-U	4	141.7 ± 13.1	C–D, <.05

[a] PBS = buffered saline with sodium phosphate; TRH = thyrotropin-releasing hormone; T_4 = thyroxine; Act D = actinomycin D.

[b] Mean ± SE of the mean.

Results. Effect of TRH on the oxidation of ^{14}C-6-glucose in porcine anterior pituitary slices. TRH at the concentrations of 0.5–5.0 μg/ml markedly stimulated the oxidation of ^{14}C-6-glucose as shown in Expt 1 of Table I. However, a higher concentration of TRH, 50 μg/ml, did not stimulate the oxidation of ^{14}C-6-glucose at all. In Expt 2 of Table I, effects of TRH on the oxidation of ^{14}C-1-glucose and ^{14}C-6-glucose were compared. Effect of TRH on the oxidation of ^{14}C-6-glucose was greater than that of ^{14}C-1-glucose at a concentration of 0.5 μg/ml. At a lower concentration of 0.1 μg/ml, the oxidation of ^{14}C-6-glucose was further increased, indicating that TRH is preferentially more effective in stimulating the oxidation of the sixth carbon of glucose than the first carbon of glucose.

Effect of actinomycin D and/or thyroid hormone on TRH stimulation of glucose oxidation in anterior pituitary. Effect of actinomycin D and/or thyroxine on TRH stimulation of glucose oxidation was studied in Expt 3 of Table I. In agreement with our previous study (6), thyroxine (0.1 μg/ml) completely prevented an increase of glucose oxidation produced by TRH. When pituitary slices were preincubated with actinomycin D for 30 min and then with thyroxine for 15 min before the incubation with TRH, this inhibitory action of thyroxine on TRH-induced glucose oxidation disappeared.

Effect of TRH and DBcAMP on pituitary phospholipogenesis. When 0.1–2.5 μg/ml of TRH were present in the incubation media, dose-related increases in ^{32}P incorporation into phospholipids in porcine anterior pituitary slices were observed as shown in Fig. 1. However, a higher concentration of TRH (25 μg/ml) was less effective than 2.5 μg/ml of TRH (Expt 1 of Table II). Similarly, a high concentration of DBcAMP (10^{-3} *M*) had no effect on pituitary phospholipogenesis while a lower concentration of DBcAMP (10^{-4} *M*) significantly stimulated pituitary phospholipogenesis (Expt 2 of Table II).

Effect of thyroid hormone on TRH stimulation of pituitary phospholipogenesis. In Expts 3 and 4 of Table II, effect of thyroid hormones on TRH stimulation of pituitary phospholipogenesis was studied in a manner similar to the experiment of glucose oxidation.

TABLE II. Effect of TRH on Pituitary Phospholipogenesis *in vitro*.[a]

Expt	Groups	Addition to incubation medium (μg/ml)	No. of determinations	^{32}P incorporation into phospholipids (% of control)	p value from control
1.	A	PBS	5	100.0 ± 7.3[b]	
	B	TRH, 2.5	5	149.9 ± 19.7	<.05
	C	TRH, 25.0	5	124.7 ± 6.1	<.01
2.	A	PBS	5	100.0 ± 5.6	
	B	TRH, 2.5	5	167.2 ± 21.3	<.05
	C	DBcAMP(10^{-4} *M*)	5	139.4 ± 10.9	<.01
	D	DBcAMP(10^{-3} *M*)	5	103.3 ± 12.2	NS
3.	A	PBS	4	100.0 ± 12.3	
	B	TRH, 2.5	4	169.1 ± 26.2	<.05
	C	T_4, 1.0	4	92.3 ± 16.0	
	D	T_4, 1.0 + TRH, 2.5[c]	4	133.2 ± 28.3	B–D, NS
4.	A	PBS	5	100.0 ± 10.9	
	B	TRH, 2.5	5	165.2 ± 8.0	<.01
	C	T_3, 2.0 + TRH, 2.5[c]	5	170.1 ± 11.9	
	D	T_3, 0.2 + TRH, 2.5	5	144.0 ± 12.2	B–D, NS
	E	T_3, 0.02 + TRH, 2.5	5	179.9 ± 7.4	

[a] DBcAMP = dibutyryl cyclic AMP; T_3 = triiodothyronine. Other abbreviations are the same as in Table I.

[b] Mean ± SE of the mean.

[c] Pituitary slices were preincubated with T_4 or T_3 for 30 min.

In Expt 3 of Table II, thyroxine added to the preincubation and incubation media at a concentration of 1.0 μg/ml did not significantly inhibit TRH-induced pituitary phospholipogenesis. Thyroxine alone added to the incubation media did not influence the basal rate of ^{32}P incorporation into pituitary phospholipids. In Expt 4 of Table II, effect of various concentrations of triiodothyronine on TRH-induced phospholipogenesis was studied. No inhibitory effect of triiodothyronine on TRH-induced phospholipogenesis was found, however.

Discussion. In agreement with our previous report (6), TRH stimulated glucose oxidation by the anterior pituitary and this increase of glucose oxidation was blocked by thyroid hormone. Since TRH augments TSH secretion from the pituitary (3, 4) and this TRH effect is blocked by thyroid hormone (8), and since TRH effect on the pituitary is dependent upon energy metabolism (9), it is likely that an increased secretion of TSH produced by TRH is in some way linked with an enhancement of glucose oxidation. However, this suggested sequence of events is somewhat complicated by species specificity. Pittman, Duvosky and Beschi (5) found that TRH stimulated C-6 labeled glucose but not C-1 labeled glucose in rat pituitary, while Yamamoto *et al.* (6) found that TRH stimulated C-1 labeled glucose and uniformly labeled glucose by the porcine anterior pituitary. Our present study clearly indicates that TRH can stimulate the oxidation of both C-1 and C-6 labeled glucose in porcine anterior pituitary, and further that TRH stimulation of glucose oxidation is apparently greater on C-6 than on C-1 labeled glucose. Thus, as far as C-6 labeled glucose is concerned, it seems established that TRH stimulates the oxidation of glucose by the anterior

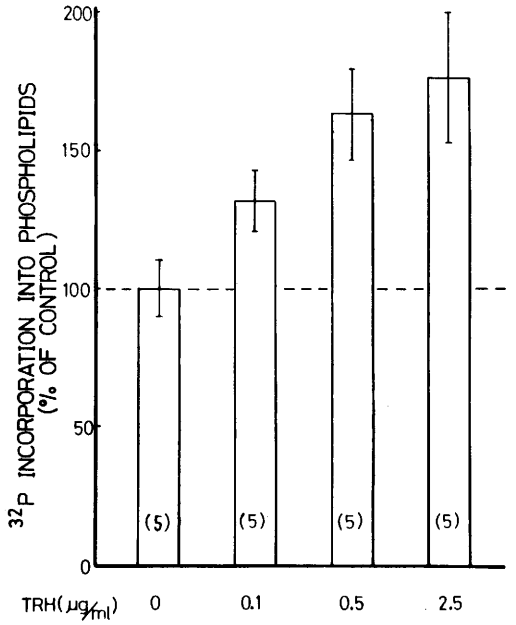

FIG. 1. Effect of various concentrations of TRH on pituitary phospholipogenesis. Bars and vertical lines represent mean ± standard error of the mean. Number of determinations given in parentheses. TRH (0.1 μg/ml) significantly stimulated ^{32}P incorporation into pituitary phospholipids with p value less than 0.05.

pituitary while TRH is stimulating TSH secretion. This situation is further studied by using actinomycin D, since thyroxine fails to depress an increase of TSH release produced by TRH in the presence of actinomycin D (10). In accordance with TSH data (10), thyroxine fails to depress an increase of glucose oxidation by the anterior pituitary produced by TRH in the presence of actinomycin D. Thus the manifestation or inhibition of TRH effect on pituitary TSH release correlates well with an increase or decrease of glucose oxidation by the anterior pituitary under several experimental conditions. These data strongly suggest that an increase of glucose oxidation is closely linked with the TSH-releasing action of TRH.

Theoretically it is possible that structural alteration of cell membranes of the anterior pituitary in response to TRH may be followed by an increase of phospholipid synthesis, but this possibility has not been studied to date. In support of our speculation, TRH apparently stimulates phospholipid synthesis by the anterior pituitary, the magnitude of increase being related with the dose of TRH administered. Also DBcAMP produces an increase of phospholipid synthesis by the anterior pituitary. Since TRH stimulates synthesis of 3′,5′-cyclic AMP by the anterior pituitary (11), it seems that TRH stimulates phospholipid synthesis through an increase of cyclic AMP, the second messenger of TRH. However, interpretation of this phospholipid synthesis in terms of pituitary–thyroid feedback control is difficult, since a large dose of triiodothyronine fails to depress phospholipid synthesis produced by TRH.

Summary. Synthetic TRH stimulated the oxidation of ^{14}C-1-glucose and ^{14}C-6-glucose to $^{14}CO_2$ in porcine anterior pituitary slices. This effect of TRH on the oxidation of ^{14}C-6-glucose was greater than that of ^{14}C-1-glucose. Thyroxine blocked the increase of glucose oxidation produced by TRH, but failed to do so in the presence of actinomycin D. Synthetic TRH also stimulated ^{32}P incorporation into phospholipids, the magnitude of stimulation being related to the dose of TRH used. DBcAMP also stimulated phospholipogenesis by the anterior pituitary. TRH stimulation of pituitary phospholipogenesis was not inhibited by triiodothyronine.

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Received Sept. 26, 1972. P.S.E.B.M., 1973, Vol. 142.