Stimulation by Synthetic Thyrotropin-Releasing Hormone of Glucose Oxidation in Porcine and Rat Pituitary Slices (36529)

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(Introduced by M. A. Greer)

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The mechanism of action of thyrotropin-releasing hormone (TRH) by which thyrotropin (TSH) secretion by the pituitary occurs is not known. Since structural alteration of cell membranes in target glands in response to tropic hormones is known to be the energy-requiring step, not only in the transport process but in the emiocytotic discharge of secretion from the cell as well (1), and since TSH secretion by the pituitary induced by TRH is also known to be dependent on energy metabolism (2), it is likely that the secretory process in the pituitary stimulated by TRH is in some way linked to enhancement of glucose oxidation. While our study was in progress, Pittman, Dubovsky and Beschi (3) have reported that synthetic TRH stimulated oxidation of glucose-6-^{14}C to $^{14}CO_2$ but not oxidation of glucose-1-^{14}C (C-1) in the rat pituitary.

In the present study, however, we have demonstrated that TRH stimulated the oxidation of C-1 and uniformly labeled glucose in porcine anterior pituitary slices, and that such TRH effect was prevented by the addition of thyroxine to the incubation medium.

Materials and Methods. Synthetic TRH was kindly donated by the Abbott Laboratories, Japan. TRH was dissolved in saline buffered with 0.01 *M* sodium phosphate (pH 7.4) (PBS) just before use. The sodium salt of thyroxine was dissolved in 0.1 *N* sodium hydroxide, diluted with PBS and added to preincubation and incubation media at a concentration of 10 μg/ml. 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 lobes, 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 serving as a control and other as an experimental. Slices were lightly blotted on filter paper and weighed. Then, each slice was placed in a 15-ml Erlenmeyer flask containing 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4), 0.5 μCi of glucose-1-^{14}C or of glucose-U-^{14}C (New England Nuclear Corp.), 1.0 mg of glucose, and the test substances. The flasks were gassed with 95% O_2–5% CO_2 and incubated in a Dubnoff metabolic shaker at 37° for 60 min except in Expt. 2, where the incubation was performed for 30–120 min. At the end of the incubation, 0.3 ml of Hyamine base was injected through the rubber cap into the center well and the reaction was stopped by the addition of 0.2 ml of 10 *N* H_2SO_4 to the medium. After shaking for an additional 60 min, the Hyamine was transferred to a counting vial. The center well was rinsed with 0.4% diphenyloxazole-in-toluene and the volume in the counting vial was made up to 10 ml with 0.4% diphenyloxazole-in-toluene and counted in an Aloka liquid scintillation counter.

Eight male rats of Wistar strain, weighing about 200 g, served as pituitary donors in Expts. 3 and 4. After removal, each pituitary was cut in half. Three to five pituitary halves were weighed together and then placed in each of two flasks, one serving as a control and the other with an equal number of pituitary halves from the same animals, as an experimental. In an experiment where animals were pretreated (iv) with saline for control or TRH for experimental, three hemipituitaries from different animals were used for one flask. The values of $^{14}CO_2$ produced in control flasks were usually 50–150

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

Expt.	Groups	Pituitary slices	Addition to incubation medium (μg/ml)	No. of determinations	$^{14}CO_2$ produced (% of control)	p value from control
1.	A	Porcine, anterior	PBS	4	100.0 ± 4.5[a]	
	B	Porcine, anterior	TRH, 1.25	4	117.5 ± 7.3	NS
	C	Porcine, anterior	TRH, 2.5	5	121.6 ± 3.2	$p < .001$
	D	Porcine, anterior	TRH, 5.0	4	128.0 ± 6.1	$p < .01$
	E	Porcine, posterior	PBS	4	100.0 ± 7.0	
	F	Porcine, posterior	TRH, 2.5	4	87.9 ± 6.4	NS
2.	30 min	Porcine, anterior	PBS	4	100.0 ± 5.6	
	30 min	Porcine, anterior	TRH, 2.5	4	104.2 ± 6.7	NS
	60 min	Porcine, anterior	PBS	8	100.0 ± 3.5	
	60 min	Porcine, anterior	TRH, 2.5	8	123.2 ± 4.1	$p < .05$
	120 min	Porcine, anterior	PBS	4	100.0 ± 3.0	
	120 min	Porcine, anterior	TRH, 2.5	4	141.2 ± 14.2	$p < .001$
3.		Rat, anterior	PBS	10	100.0 ± 5.6	
		Rat, anterior	TRH, 2.0	10	95.5 ± 5.1	NS
4.		Rat, anterior	PBS	5	100.0 ± 4.8	
		Rat, anterior	TRH, 2.0	5	91.9 ± 6.2	NS

[a] Mean ± SE of the mean. The slices of the pituitaries, 0.5 mg thick and 60–80 mg weight, were incubated in 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 0.5 μCi of glucose-1-^{14}C, 1.0 mg of glucose and TRH for 60 min (Expt. 1 and 3) or varying periods (Expt. 2). The values of $^{14}CO_2$ produced in control flasks were 50–150 cpm/mg/hr and those in experimentals were expressed as percentage of the control. In Expt. 4, 8 animals were divided into two groups and 1.0 μg of TRH or saline was injected intravenously 10 min before sacrifice and the pituitaries were incubated further in the presence or absence of TRH. PBS = saline buffered with 0.01 *M* sodium phosphate (pH 7.4). TRH (1.25 μg/ml) = final concentration of TRH in the incubation medium was 1.25 μg/ml.

cpm/mg/hr and those in experimentals were always expressed as percentage of control. Statistical analysis was carried out by the Student's *t* test.

Results. When 2.5–5.0 μg/ml of TRH were present in the incubation medium, the oxidation of glucose-1-^{14}C to $^{14}CO_2$ in the porcine anterior pituitary increased significantly (Expt. 1C and D of Table I). However, a similar amount of TRH failed to augment glucose oxidation in the posterior pituitary (Expt. 1F of Table I). As shown in Expt. 2 of Table I, this stimulation of glucose oxidation increased progressively with time.

In order to test species specificity in pituitary response to TRH, the effect of *in vitro* administration of TRH (2.0 μg/ml) on glucose (glucose-1-^{14}C) oxidation in rat hemipituitaries was studied (Expt. 3 of Table I). No increase of glucose oxidation was found, however. In the second step, 1.0 μg TRH was administered iv and, 10 min later, the pituitaries were removed for *in vitro* analysis of glucose oxidation. As expected (4), this dose of TRH stimulated pituitary activity as evidenced by the fact that the number of intrathyroidal colloid droplets increased markedly (400 droplets in TRH and 20 droplets in control/25 follicles). These pituitaries were further incubated *in vitro* in the presence of TRH (2.0 μg/ml). In spite of these treatments, no increase of glucose oxidation in the rat anterior pituitary was found (Expt. 4 of Table I).

In the final experiment, the effect of thyroxine on increased glucose oxidation produced by TRH was studied using porcine anterior pituitary. The presence of TRH (2.5

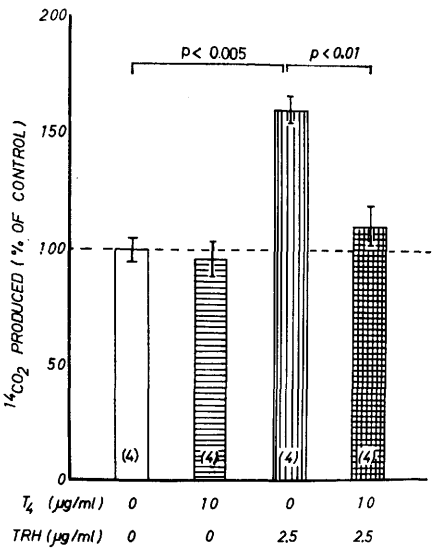

FIG. 1. Effect of thyroxine (T_4) on TRH-stimulated production of $^{14}CO_2$ from glucose-U-^{14}C in porcine anterior pituitary slices. Slices were preincubated for 30 min in the presence or absence of T_4 before addition of TRH and then incubated for 1 hr. Bars and vertical lines represent mean and ± standard error of the mean. Number of determinations given in parentheses. Incubation procedures were the same as in Table I.

μg/ml) significantly augmented the oxidation of glucose-U-^{14}C to $^{14}CO_2$. This effect of TRH was inhibited when 10 μg/ml of thyroxine was present in the incubation medium (Fig. 1).

Discussion. Considerable evidence has accumulated that synthetic TRH stimulates TSH release from the pituitary *in vivo* and *in vitro* (5, 6). However, alteration in the patterns of intermediary metabolism in the pituitary caused by TRH has not been well investigated. Migliorini and Antunes-Rodrigues (7) have recently reported that hypothalamic extract stimulated pituitary glucose oxidation. Other agents such as epinephrine have been reported to stimulate pituitary oxidation of C-1 labeled glucose (8, 9). Our present study has demonstrated the stimulatory effect of synthetic TRH on the oxidation of C-1 and uniformly labeled glucose in porcine anterior pituitary slices but not in porcine posterior pituitary slices, indicating that synthetic TRH is a specific stimulator for the anterior pituitary and that the hexose monophosphate pathway is also operating in the pituitary in the presence of tropic hormone, TRH in this case.

Bowers and Robinson (10) have recently suggested that TRH stimulates pituitary release of TSH by elevating pituitary cyclic adenosine monophosphate (cAMP), and that triiodothyronine blocks TRH effect by stimulating cAMP phosphodiesterase. Interestingly, our present study indicates that an increase of glucose oxidation in porcine anterior pituitary by TRH was almost completely blocked by thyroxine added to the incubation media. These findings seem to support the idea that an increase of pituitary glucose oxidation is in some way related to the manifestation of TRH effect on TSH release from the pituitary (9). However, our findings in rats may be against the above hypothesis, since *in vitro* addition of TRH failed to stimulate the oxidation of glucose-1-^{14}C in rat pituitaries, whether the pituitaries were obtained from untreated rats or rats pretreated with a large dose of TRH. While our study was in progress, however, Pittman, Dubovsky and Beschi (3) made an observation that synthetic TRH stimulated the oxidation of glucose-6-^{14}C without affecting the metabolism of glucose-1-^{14}C in rat pituitaries. Therefore, these findings led us to conclude that TRH augments pituitary release of TSH on one hand and glucose oxidation in the pituitary on the other hand. Further experiments are required to determine whether or not an increase of glucose oxidation in the pituitary is prerequisite for the manifestation of TRH effect on TSH release.

Summary. Synthetic TRH stimulated the oxidation of glucose-1-^{14}C to $^{14}CO_2$ at a concentration of 2.5 μg/ml in porcine anterior pituitary slices. This stimulation was not observed in porcine posterior pituitary slices or in rat hemipituitaries. TRH also stimulated the production of $^{14}CO_2$ from glucose-U-^{14}C in the porcine anterior pituitary. This stimulatory effect of TRH on glucose oxidation was blocked by thyroxine added to the incubation medium at a concentration of 10 μg/ml.

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