Tuftsin stimulates thyrotropin secretion in rats

T. Mitsuma, T. Nogimori and M. Chaya

Fourth Department of Internal Medicine, Aichi Medical University, Nagakute, Aichi 480-11 (Japan), 13 January 1984

Summary. Tuftsin acts at the hypothalamus level to stimulate thyrotropin-releasing hormone and thyrotropin secretion in rats.
Key words. Tuftsin; thyrotropin; thyrotropin-releasing hormone.

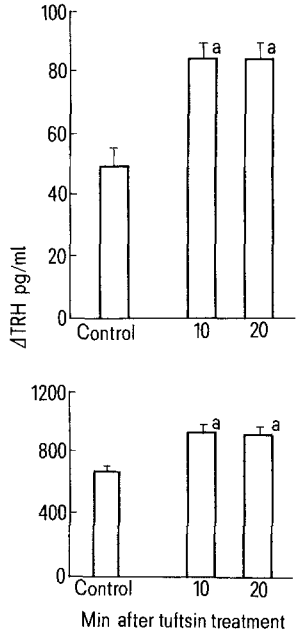
Tuftsin was discovered and isolated by Najjar et al.[1] on the basis of its ability to stimulate the phagocytic activity of polymorphonuclear granulocytes[1,2]. It is found in γ-globulin and produced by the spleen[2]. Recently, Onaya et al.[3] reported that tuftsin potentiated thyrotropin-induced thyroid hormone release in vitro. However, the effects of tuftsin on thyrotropin (TSH) secretion have not been reported to date. The present authors have therefore investigated the effects of tuftsin on TSH and thyrotropin-releasing hormone (TRH) in rats.

Materials and methods. Animals: Male rats (Wistar strain) weighing 200 g and housed in temperature-(22 °C) and humidity-(60%) controlled quarters with illumination from 06.00–18.00 h, were given a diet of laboratory chow and water ad libitum. Drugs: Synthetic TRH and tuftsin were obtained from the Protein Research Foundation (Japan).

Experimental design: All experiments were conducted in a temperature-controlled room (22 °C). Rats were divided into three groups and all were sacrificed between 13.00 and 15.00 h to prevent diurnal variation. Group 1 (42 rats) received tuftsin (300 µg/kg) i.v.: seven rats were decapitated at 10, 20, 30, 40, 50 and 60 min under ether anesthesia after the injection. Trunk blood was collected in heparinized tubes kept on ice. The hypothalami were obtained by the method previously described[4]. In group 2, 10–20 min after tuftsin injection, the rats were placed in a room maintained at 4 °C and decapitated after 45 min. In group 3, 10–20 min after tuftsin injection, the rats were given 1 µg of synthetic TRH i.v. Exactly 10 min later, they were decapitated. For the control, saline was injected.

Method of measurement of inactivation of TRH immunoreactivity by plasma or hypothalamus in vitro: 10 ng of synthetic TRH was added to 1.0 ml of plasma or 10 mg of homogenized hypothalami (in 1.0 ml 0.01 M phosphate buffer, pH 7.4) at 4 °C and the mixture incubated at 37 °C for 30 min, after which 5.0 ml of cold methanol was added and the mixture was centrifuged at 4 °C. The resultant supernatants were dried and resuspended in 0.01 M phosphate buffer (pH 7.4). The recovery of TRH added was measured by radioimmunoassay[5], and the results were expressed as a percentage of TRH added. Assay methods: TRH, thyroxine (T_4) and 3,3',5-triiodothyronine (T_3) were measured by radioimmunoassay[5,6]. TSH was determined by a NIAMDD rat TSH radioimmunoassay kit[7]. TRH content in the hypothalamus was expressed as the amount per total dissected hypothalamic section. Statistics: Mean and standard error of the mean were calculated for each group. Student's t-test was used to evaluate the differences between the control and experimental groups.

Results. The hypothalamic immunoreactive TRH (ir-TRH) content decreased significantly after tuftsin injection, whereas its plasma concentration tended to increase, but not significantly (table). Plasma TSH levels increased significantly above



Effects of tuftsin treatment on plasma immunoreactive TRH and TSH responses to cold (4 °C). Values are expressed as the mean ± SE in each group of 7 rats. Δ TRH and Δ TSH indicated as the increment of plasma immunoreactive TRH and TSH after 45 min cold exposure. Differences from the control are shown by a $p < 0.001$.

after drug administration	0	10	20	30	40	50	60 min.
control							
TRH contents in the hypothalamus (ng)	4.1 ± 0.2	4.2 ± 0.3	4.3 ± 0.3	4.2 ± 0.2	4.1 ± 0.2	4.2 ± 0.3	4.0 ± 0.3
TRH in plasma (pg/ml)	5.2 ± 1.9	5.1 ± 2.0	6.0 ± 1.8	6.4 ± 1.9	6.0 ± 1.9	6.5 ± 2.0	5.8 ± 1.7
TSH in plasma (ng/ml)	258 ± 24	255 ± 25	252 ± 24	260 ± 25	258 ± 24	260 ± 26	265 ± 25
T_4 in plasma (µg/dl)	5.1 ± 0.3	4.8 ± 0.4	5.0 ± 0.3	5.2 ± 0.4	4.9 ± 0.3	4.9 ± 0.3	5.0 ± 0.4
T_3 in plasma (ng/dl)	52 ± 2.8	50 ± 2.8	49 ± 3.0	48 ± 2.6	50 ± 2.7	51 ± 2.5	52 ± 2.9
tuftsin							
TRH contnts in the hypothalamus (ng)		4.0 ± 0.3	3.8 ± 0.2	3.5 ± 0.2[b]	3.4 ± 0.2[b]	3.6 ± 0.3	3.7 ± 0.3
TRH in plasma (pg/ml)		6.8 ± 2.0	9.1 ± 2.1	11.2 ± 2.2	9.1 ± 1.9	5.0 ± 1.5	4.8 ± 1.4
TSH in plasma (ng/ml)		300 ± 25	380 ± 26[a]	410 ± 28[a]	332 ± 26	310 ± 26	280 ± 27
T_4 in plasma µg/dl		5.0 ± 0.3	5.1 ± 0.4	5.0 ± 0.3	5.2 ± 0.4	5.1 ± 0.3	5.1 ± 0.3
T_3 in plasma (ng)dl)		49 ± 2.8	50 ± 2.9	53 ± 2.5	52 ± 3.0	54 ± 2.9	54 ± 2.8

Table 1. Effects of tuftsin treatment on the hypothalamic immunoreactive TRH contents, plasma immunoreactive TRH, TSH, T_4 and T_3 levels. Values are expressed as the mean ± SE in each group of 7 rats. Differences from the control are indicated as [a] $p < 0.005$ and [b] $p < 0.05$.

200 µg/kg in a dose-related manner, with a zenith at 30 min after injection (table). Plasma thyroid hormone levels did not change significantly after tuftsin injection (table). The plasma ir-TRH and TSH responses to cold were significantly enhanced by tuftsin (fig.). The plasma TSH levels after TRH administration were 1120 ± 67 ng/ml at 10 min and 1099 ± 56 ng/ml after tuftsin injection, respectively. The inactivation of TRH immunoreactivity by plasma or hypothalamus in vitro after tuftsin injection was around 35% with plasma and 47% with hypothalamus and did not differ from that of the control.

Discussion. Tuftsin, which was isolated by Najjar et al.[1], stimulates the phagocytic activity of blood polymorphnuclear granulocytes[2]. However, the effect of tuftsin on hormone release has rarely been studied[3]. The present experiments demonstrate that tuftsin increases plasma TSH levels. The increase in plasma TSH levels might be the result of an action of tuftsin at the hypothalamus level or at the pituitary level. The present study revealed that the hypothalamic ir-TRH decreased significantly, whereas its plasma concentration tended to increase after tuftsin injection. The hypothalamic ir-TRH content and its plasma concentration may be expressed as a balance among TRH release, synthesis and degradation. The inactivation of TRH immunoreactivity by plasma or hypothalamus in vitro after tuftsin injection did not differ from that of the control, suggesting that tuftsin may well affect TRH release or synthesis. The plasma ir-TRH and TSH responses to cold, which are known to be mediated by TRH activity[8], were enhanced by tuftsin. It has been reported that plasma TSH levels were significantly elevated after 15 min, peaked after 45 min cold exposure, and remained significantly elevated for up to 4 h[9]. Therefore, plasma TSH levels were observed at 45 min after cold exposure in the present experiment. The present investigations, taken together, suggest that tuftsin may act at the hypothalamus level to stimulate TRH release.

The plasma TSH response to TRH did not differ from that of the control, indicating that tuftsin may not act at the pituitary level. Since plasma thyroid hormone levels did not change after tuftsin injection, tuftsin may not act at the thyroid gland. However, thyroid hormone metabolism is very slow. Moreover, Onaya et al.[3] reported that tuftsin potentiated TSH-induced thyroid hormone secretion. Thus, a longer observation period is necessary to verify this point.

These findings suggest that tuftsin acts at the hypothalamus level to stimulate TRH release in rats.

1 Najjar, V.A., and Nishioka, K., Nature *228* (1970) 672.
2 Nishioka, K., Constantopoulos, A., Satok, P.S., and Najjar, V.A., Biochem. biophys. Res. Commun. *47* (1972) 172.
3 Onaya, T., Komiya, I., and Hashizume, K., Thyroid Research VIII, p.85. Eds J.R. Stockigt and S. Nagataki. Australian Academy of Science, Canberra 1980.
4 Mitsuma, T., Hirooka, Y., and Nihei, N., Endocr. jap. *51* (1976) 806.
5 Mitsuma, T., Hirooka, Y., and Nihei, N., Acta endocr., Copenh. *83* (1976) 225.
6 Mitsuma, T., Colucci, J., Shenkman, L., and Hollander, C.S., Biochem. biophys. Res. Commun. *51* (1972) 21107.
7 Rat TSH radioimmunoassay kit was kindly suppled by NIAMDD.
8 Szabo, M., and Frohman, L.A., Endocrinology *101* (1977) 1023.
9 Jobin, M., Ferland, L., Cote, J., and Labtie, F., Neuroendocrinology *18* (1975) 204.

0014-4754/85/010113-02$1.50 + 0.20/0

Bovine pituitary intraglandular colloid fraction F_5 localized in the rat endocrine pancreas[1]

W.H. Boyd[2]

Section of Human Anatomy, Department of Biomedical Sciences, University of Guelph, Guelph (Ontario, Canada N1G 2W1), 20 March 1984

Summary. Bovine pituitary intraglandular colloid thought to be a waste product, is the holocrine secretion of intermediate lobe cells. It is housed in the intraglandular lumen (residual lumen) and is extruded into the venous circulation of the cavernous sinuses via clefts in the capsule of the gland aligned with the intraglandular lumen. Intraglandular colloid, fraction F_5 (mol.wt 34,000), radiolabeled with (^{125}I)Na and injected (0.15 ml) into the right internal jugular vein of male Wistar rats, accumulated in the endocrine pancreas. Autoradiographs showed that the material had specifically localized in the capillary network of the endocrine pancreas. Since the intermediate lobe is poorly vascularized, intraglandular colloid is considered to be the transport medium for intermediate lobe materials.

Key words. Transport; pituitary; pancreas; intraglandular colloid; radiolabeling; autoradiography.

It is not clear how intermediate lobe materials, i.e., the ACTH/βLPH family of peptides produced by intermediate lobe cells gain the systemic circulation since the lobe is poorly vascularized[3]. In addition there is some question regarding the final post-translational modification of these peptides, the form(s) in which they leave the cell(s) and the route(s) by which they reach the circulation. The post-translational processing of peptides within the intermediate lobe cell(s) (adrenocorticotropic hormone (ACTH), α-melanotrophin (α-MSH) and ACTH (18–39)-like peptides, β-lipotropin (β-LPH), β-endorphin (β-EP)-like and β LPH-like peptides)[4–6], suggests high levels of cellular activity. Studies of a large series of mammalian pituitary glands, showed that the bovine intermediate lobe best reflects this activity and is thus being used as a model in these studies. There is a constant breakdown of cells in the marginal half of the intermediate lobe resulting in the formation of intraglandular colloid, the holocrine secretion of intermediate lobe cells housed in the intraglandular lumen (residual lumen). The intraglandular colloid appears in the form of either a clear fluid, semifluid or gel. The marginal layer of intermediate lobe cells is restored by the direct division of cells in the deep layer of the intermediate lobe[7,8]. Clefts in the capsule of the gland aligned with the intraglandular lumen, provide an efficient entrance of intraglandular colloid into the venous circulation of the cavernous sinuses[8,9]. Indeed, intraglandular colloid is usually thought to be a waste product[10] and has been over-