RAPID COMMUNICATION

Central Nervous System Mediated Stimulation by Thyrotropin-Releasing Hormone of Microcirculation in Thyroid Gland of Rats

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Synopsis

The blood flow of thyroid, adrenal cortex and renal cortex in the pentobarbital anesthetized rat was assessed from hydrogen gas desaturation curve. The microcirculation of thyroid was markedly augmented within 2 min after an intraventricular injection of Thyrotropin-Releasing Hormone (TRH) while Met-Enkephalin (ENK) failed to influence. Both TRH and ENK stimulated the microcirculation of adrenal cortex moderately. ENK diminished the microcirculation of renal cortex whereas TRH did not exert any effect. The response of thyroid to TRH was abolished by vagotomy, thus the existence of a specific TRH-vagus -thyroid connection was indicated.

It is now well recognized that thyrotropin-releasing hormone (TRH) directly affects the central nervous system (CNS) to produce a number of physiological and behavioral reactions. On this basis Guillemin (1977) proposed a hypothesis that TRH and other hypothalamic peptides might modify the activity of neuronal circuit in the CNS. The present author has demonstrated the alteration by TRH of visual evoked potential in the reticular formation suggesting an effect of TRH on the excitability of the neuronal network (Tonoue, 1977). Recently we have found that an intracerebroventricular (i.c.v.) injection of TRH stimulates the vagal efferents which control the electromyographic activity of the duodenum in the rat (Tonoue and Nomoto, 1979). This finding led us to consider that TRH in the CNS is capable of modifying the activity of the specific autonomic nerve efferents. On the other hand, the effects of epinephrine

upon the thyroid have been extensively studied and shown to diminish thyroid blood flow (Ackerman and Arons, 1958; Mowbray and Peart, 1960; Falconer, 1967; Ahn *et al.*, 1969). Contrary, acetylcholine is known to enhance thyroid blood flow (Söderberg 1958, 1959). It has been reported that the thyroid blood flow is augmented within a few minutes after exposing the rat to ether vapor (Goldman, 1963). More recently blood capillary enlargement during the development of thyroid hyperplasia has also been demonstrated (Wollman *et al.*, 1978). These previous studies indicate that the thyroid blood flow is controlled by the CNS through the autonomic nervous system. We report here the marked elevation of local blood flow of the thyroid immediately after an i.c.v. injection of TRH and the dependence of this response on the vagal innervation.

Materials and Methods

Wistar-Imamichi male rats weighing 350–450 g were used. The animals were kept in a temperature controlled ($23 \pm 2^\circ\text{C}$) and artificially illuminated (0600–2000 light) room and maintained on pellets and water *ad libitum*. They were anesthetized by an i.p. injection of pentobarbital (50 mg/kg) and the tissue blood flow was assessed from the desaturation curve of hydrogen gas adopting the method developed by Aukland *et al.* (1964) and modified by Neely *et al.* (1965). Briefly, the current generated by oxidation of molecular hydrogen to hydrogen ions at the surface of a platinum electrode which was inserted into the tissue was measured after the inhalation of air containing 10% hydrogen. The hydrogen electrode consisted of a platinum wire (0.095 mm in diameter) insulated except 1 mm tip which was coated with platinum black by immersing it as the cathode in a 3% solution of platinum chloride. The electrode tip was introduced into the tissue 1.5 mm beneath the surface of the organ. The circuit (Unique Medical Co. PHG-200) used to connect the electrode to a recorder was depicted elsewhere and is ready for distribution on request. The reference electrode was an Ag-AgCl wire placed under the skin on the back. The desaturation curve of the tissue hydrogen was recorded after the cessation of inhaling hydrogen gas. From the half-time of decay in the tissue hydrogen the blood flow rate was calculated according to the formula of Kety (1951): Blood flow ($\text{ml}/\text{min}/100 \text{ g}$) = $69.3/\text{half-time (min)}$. Injection into the lateral ventricle was performed by the method of Noble *et al.* (1967) just at the cessation of inhalation of hydrogen gas. TRH (Takeda Chem. Ind.) or $[\text{Met}^5]$ -Enkephalin (ENK, Protein Research Foundation, Osaka) was dissolved in physiological saline and injected in a dose of 2 nmoles/ $2 \mu\text{l}/100 \text{ g}$. Vagotomy was performed ipsilaterally by cutting the vagus at the superior extreme of the cervix. Sympathectomy was performed by removing the ipsilateral superior cervical ganglion.

Results and Discussion

All the desaturation curves obtained in this study were biphasic (Fig. 1). The multi-exponential decay seems to reflect the inhomogeneity of blood flow in the area surrounding the electrode (Aukland *et al.*, 1964). The initial fast component reflects, in general, the high blood flow rate in the large blood vessels in the tissue, however,

it exceeded the range of flows measurable by this method (Aukland *et al.*, 1964). On the other hand, the late slowest component reflects correctly the microcirculation of the area surrounding the electrode because at this phase the arterial hydrogen pressure is essentially zero and there are no factors to affect the hydrogen decay in the tissue other than the circulation rate. In this report the term microcirculation expresses the blood flow rate calculated from the half-time of the slowest component of hydrogen decay. In Fig. 1 are shown the semilogarithmic plots of hydrogen desaturation curves obtained in the same site at successive intervals. By triplicate preliminary experiments of the same type it was confirmed that neither fast nor slow component of hydrogen decay changed spontaneously during the experiment in all the tissues studied.

An i.c.v. injection of TRH augmented within 2 min the microcirculation in the thyroid whereas ENK did not exert any effect (Fig. 2 and Table 1). Since an intravenous injection of TSH did not affect thyroid blood flow (Fig. 1 and Table 1) the effect of TRH was not due to the pituitary TSH secretion. The effect of TSH on the thyroidal blood flow has been reported to occur much later (Melandar *et al.*, 1975). In the adrenal cortex, microcirculation was augmented by either i.c.v. TRH or ENK, though the extent of response was moderate. Contrary, in the renal cortex i.c.v. injected ENK diminished but TRH did not alter the microcirculation (Table 1).

In the rats which received acute vagotomy an i.c.v. injection of TRH failed to augment the thyroid microcirculation, the whereas removing of the superior cervical ganglion, sympathectomy, did not abolish the response to TRH (Table 1). The extent of the response was lower in the sympathectomized rat than in the normal rat. It is likely that the acute manipulation of ganglion elicited the thyroidal vascular response

Fig. 1. Hydrogen desaturation curves in the renal cortex and thyroid of the pentobarbital anesthetized rat. Current due to hydrogen oxidation is plotted in arbitrary units on a logarithmic scale. Scale of abscissa, 10 sec. Measurements were carried out at 10 min intervals in the renal cortex (Top); before and 1, 5 and 15 min after an i.v. injection of TSH (Thyropar, Armour, 200 mu) in the thyroid (Bottom). Numbers beside curves are half-time (sec) of decay.

Fig. 2. Hydrogen desaturation curves in the thyroid before and immediately after an i.c.v. injection of TRH or ENK (2 nmoles/100 g). Current due to hydrogen oxidation is plotted in arbitrary units on a logarithmic scale. Scale of abscissa, 10 sec.

Table 1. Effects of intraventricular injection of TRH or Met-Enkephalin on the microcirculation of thyroid, adrenal cortex and renal cortex of the rat.

Organ	Peptide (2 nmoles/100 g)	Microcirculation (ml/min/100 g)			%
		Before	After	Difference	
Thyroid (5)	Saline	75.9 ± 6.0	75.7 ± 7.2	-0.2 ± 1.5	-0.3
Thyroid (6)	TRH	80.1 ± 14.1	159.2 ± 21.3	79.1 ± 10.0**	98.8
Thyroid (4)	ENK	69.5 ± 8.0	70.5 ± 6.6	1.0 ± 1.5	1.4
Thyroid (4)	TSH ^a	72.1 ± 2.8	69.7 ± 2.5	-2.5 ± 2.5	-3.5
Adrenal (4)	Saline	69.5 ± 5.9	69.5 ± 5.2	0.0 ± 1.2	0
Adrenal (4)	TRH	68.5 ± 5.2	88.2 ± 3.5	19.7 ± 3.8*	28.8
Adrenal (5)	ENK	77.8 ± 4.0	95.8 ± 8.4	18.0 ± 5.9*	23.1
Kidney (3)	Saline	93.3 ± 1.3	95.9 ± 0.7	2.6 ± 1.8	2.9
Kidney (6)	TRH	94.6 ± 1.7	92.5 ± 4.4	-1.2 ± 4.4	-1.3
Kidney (4)	ENK	93.9 ± 9.8	86.6 ± 9.3	-7.1 ± 1.2**	-7.6
Thyroid (5)	TRH	69.7 ± 6.3	61.3 ± 5.3	-8.4 ± 3.6	-12.1
(Vagotomy)					
Thyroid (4)	TRH	89.7 ± 2.6	118.8 ± 5.5	28.9 ± 5.5*	32.3
(Sympathectomy)					

Mean ± S.E. (), No. of rats. ** $p < 0.01$, * $p < 0.05$ in paired t -test.

a, intravenous injection (200 mu/rat).

which counteracted the vasodilation.

The finding in this study indicates that TRH administered into the brain stimulates the vagal efferents involved in the regulation of thyroidal microcirculation. The rat thyroid blood flow assessed by ^{86}Rb uptake has been reported to be enhanced immediately after the administration of serotonin and histamine or their liberator (Melander *et al.*, 1975). In the submaxillary gland a model has been proposed that acetylcholine from parasympathetic nerve ending releases histamine from the "glandrop" histamine store (Lorenz *et al.*, 1968). Very recently the presence of cholinergic nerves upon in the murine thyroid and their influences on not only thyroid blood flow but also hormone secretion were reported (Melander and Sundler, 1979). Thus, tentatively a scheme may be considered as follows: TRH in the CNS affects the neuronal circuit to activate the specific vagal efferents which stimulate in the thyroid the histamine and/or serotonin liberation resulting in the vasodilatation.

The central effects of TRH or ENK on the microcirculation of adrenal or renal cortex were also observed. In this study, however, they serve as showing that the central effect of TRH on the thyroid has specificity to some extent. Further studies are required to reveal the central effects of neuropeptides on the microcirculation in the various endocrine organs. The present finding together with our previous studies

on the CNS mediated effects of TRH on the heart rate (Tonoue, 1977) or digestive tract (Tonoue and Nomoto, 1979) seems to suggest the involvement of TRH in the regulation of the specific, to some extent, vagal system.

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