Effects of eledoisin on thyrotrrophin secretion in rats

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Abstract. The effect of peripheral administration of eledoisin on thyrotrophin-releasing hormone (TRH) and thyrotrrophin (TSH) secretion in rats were studied. Eledoisin (500 mg/kg) was injected iv, and the rats were serially decapitated. TRH, TSH and thyroid hormone were measured by radioimmunoassay. The hypothalamic immunoreactive TRH (ir-TRH) content increased significantly after eledoisin injection, whereas its plasma concentration tended to decrease, but not significantly. Plasma TSH levels decreased significantly in a dose-related manner with a nadir at 40 min after the injection. Plasma thyroid hormone levels did not change significantly. Plasma ir-TRH and TSH responses to cold were inhibited by eledoisin, but the plasma TSH response to TRH was not affected. In the pimozide- or para-chlorophenylaniline-pretreated group, the inhibitory effect of eledoisin on TSH levels was prevented, but not in the L-dopa- or 5-hydroxytryptophan-pretreated group. These drugs alone did not affect plasma TSH levels at the dose used. The inactivation of TRH immunoreactivity by plasma or hypothalalus in vitro after eledoisin injection did not differ from that of controls.

These findings suggest that eledoisin acts on the hypothalalus to inhibit TRH release, and its effects are modified by amines of the central nervous system.

Eledoisin was isolated from the posterior salivary glands of the Mediterranean octopod Eledone moschata (Erspamer & Anastasi 1962) and is one of tachykinin (Erspamer 1981). Eledoisin potently stimulates intestinal and gastro-urinary smooth muscle and, when injected intravenicularly, induces elevation of vasopressin levels (Erspamer 1981; Cantalamessa et al. 1982). We previously reported that substance P, one element of tachykinin, inhibited thyrotrrophin (TSH) secretion in rats (Mitsuma & Nogimori 1984). However, effects of eledoisin on thyrotrophin-releasing hormone (TRH) and TSH secretion have not been reported. Therefore, the authors investigated the effect of eledoisin on TRH and TSH secretion in rats and the possible mechanism of eledoisin's effect on TRH and TSH secretion.

Materials and Methods

Animals

Male rats (Wistar strain), weighing 200 g housed in temperature (22°C)- and humidity (60%)-controlled quarters with lights on from 6.00 to 18.00h, were given a diet of laboratory chow and water ad libitum.

Drugs

Synthetic TRH was obtained from the Protein Research Foundation (Japan). Synthetic eledoisin was purchased from the Funakosi Pharm. Co., Ltd. (Japan). L-dopa was kindly supplied from Sankyo Co., Ltd. (Japan) and pimozide by Fujisawa Pharm. Co., Ltd. (Japan). Para-chlorophenylaniline (PCPA) and 5-hydroxytryptophan (5-HTP) were purchased from Sigma Chem. (USA). The following drugs were dissolved in 0.5 ml of saline, 0.01 N NaOH or 0.01 N HCl, and injected ip: 50 mg/kg of L-dopa (1 h before eledoisin), 10 mg/kg of 5-HTP (1 h before eledoisin), 1.0 mg/kg of pimozide (2 h before eledoisin) or 200 mg/kg of PCPA (2 h before eledoisin).

Experimental design

All experiments were conducted in a temperature-controlled room (22°C). The rats were divided into six
groups and sacrificed between 1 to 3 p.m. to prevent diurnal variation of hormones. Group 1, composed of 30 rats, was given eledoisin (500 μg/kg) iv and 5 rats in each subgroup were decapitated by guillotine without anesthesia at 10, 20, 30, 40, 50 and 60 min after the injection. Trunk blood was collected in heparinized tubes kept on ice. The hypothalami were obtained by the method previously described (Mitsuma et al. 1976a). In group 2, 0–600 μg/kg of eledoisin was administered to the rats, and 5 rats in each subgroup were decapitated at 40 min after the injection. In group 3, 10–20 min after eledoisin injection, the rats were placed in a room maintained at 4°C for 45 min and 5 rats in each subgroup were decapitated. In group 4, 10–20 min after eledoisin injection, the rats received 1 μg of synthetic TRH iv. Exactly 10 min later, 5 rats in each subgroup were decapitated. In group 5, the rats were pretreated with 1-dopa, pimozide, 5-HTP, PCPA or vehicle (5 at a time) and then decapitated 40 min after eledoisin injection. In group 6, the rats (5 in each subgroup) were administered saline and decapitated.

Method for measurement of inactivation of TRH immunoreactivity by plasma or hypothalamus

Ten ng of synthetic TRH was added to 1.0 ml of plasma or 10 ng of homogenated hypothalami (in 1.0 ml 0.01 M phosphate buffer, pH 7.4) at 4°C and then incubated at 37°C for 30 min, after which 5.0 ml of cold methanol was added and the mixture was centrifuged for 15 min 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 (Mitsuma et al. 1976b), and the results were expressed as a per cent of TRH added.

Assay methods

TRH, thyroxine (T4) and 3,3′-triiodothyronine (T3) were measured by radioimmunoassay (Mitsuma et al. 1972, 1976b). The elution profile on Sephadex G-10 of methanol-extracted rat serum after cold stimulation, containing of 5 ng of immunoreactive TRH (ir-TRH) was identical to that of synthetic TRH. TRH content in the hypothalamus is expressed as the total amount of dissected hypothalami.

Statistics

Mean and standard error of means were calculated for each group. Student’s t-test was used to evaluate the differences between the control and experimental groups.

Results

The hypothalamic ir-TRH content increased significantly after eledoisin injection, whereas its plasma concentration tended to decrease, but not

| Table 1.  |
| Effects of eledoisin treatment on immunoreactive TRH content in the hypothalamus, plasma immunoreactive TRH, TSH, T4 and T3 levels. |
| Control |
| TRH contents in the hypothalamus (ng) | 4.0 ± 0.3 | 4.0 ± 0.3 | 3.9 ± 0.3 | 3.9 ± 0.2 | 4.0 ± 0.3 | 4.1 ± 0.2 | 4.2 ± 0.3 |
| TRH in plasma (pg/ml) | 6.2 ± 1.8 | 6.0 ± 1.9 | 6.4 ± 1.7 | 6.0 ± 1.9 | 5.8 ± 1.6 | 5.6 ± 1.4 | 6.0 ± 1.9 |
| TSH in plasma (pg/ml) | 282 ± 26 | 277 ± 29 | 278 ± 27 | 284 ± 27 | 278 ± 26 | 276 ± 25 | 280 ± 26 |
| T4 in plasma (μg/dl) | 5.2 ± 0.3 | 5.0 ± 0.4 | 4.9 ± 0.3 | 5.0 ± 0.4 | 4.8 ± 0.3 | 4.9 ± 0.3 | 5.0 ± 0.4 |
| T3 in plasma (ng/dl) | 52 ± 2.9 | 49 ± 2.8 | 48 ± 2.6 | 50 ± 2.6 | 49 ± 3.0 | 51 ± 3.1 | 49 ± 2.3 |
| Eledoisin-treated |
| TRH contents in the hypothalamus (ng) | 5.2 ± 0.3b | 4.9 ± 0.2b | 4.6 ± 0.3 | 4.2 ± 0.3 | 4.1 ± 0.2 | 4.2 ± 0.4 |
| TRH in plasma (pg/ml) | 5.2 ± 1.6 | 4.2 ± 1.5 | 3.9 ± 1.6 | 5.6 ± 1.6 | 6.6 ± 1.5 | 6.5 ± 1.9 |
| TSH in plasma (ng/ml) | 250 ± 24 | 210 ± 23 | 198 ± 20b | 182 ± 17a | 220 ± 21 | 250 ± 26 |
| T4 in plasma (μg/dl) | 4.9 ± 0.4 | 4.8 ± 0.3 | 4.7 ± 0.3 | 4.7 ± 0.4 | 4.6 ± 0.2 | 4.7 ± 0.4 |
| T3 in plasma (ng/dl) | 50 ± 2.8 | 47 ± 2.5 | 45 ± 2.9 | 45 ± 2.3 | 47 ± 2.7 | 46 ± 2.6 |

Values are expressed as the mean ± SE in each group of 5 rats. Eledoisin (500 μg/kg) was administered through the tail vein. Differences from the control are indicated by a: P < 0.02 and b: P < 0.05.
Effects of eledoisin dose on plasma TSH levels. Values are expressed as the mean ± se in each group of 5 rats. Plasma TSH levels are indicated as the levels at 40 min after eledoisin injection. Differences from controls are shown by a: \( P < 0.01 \), b: \( P < 0.02 \) and c: \( P < 0.05 \). The hypothalamic ir-TRH content was 4.8 ± 0.4 ng after l-dopa injection, 3.6 ± 0.4 ng after pimozide injec-
Eledoisin, given to rats by intracerebroventricular injection produced an increase in plasma vasopressin (Cantalamessa et al. 1982). However, the effects of eledoisin on TRH and TSH secretion have not been reported. The present experiments demonstrate that eledoisin reduces plasma TSH levels in a dose-related manner, and that its effects are modified by amines of the central nervous system. The decrease in plasma TSH levels might be the result of an action of eledoisin on the hypothalamus, on the pituitary, or both.

The present study revealed that hypothalamic ir-TRH content increased significantly after eledoisin injection, whereas its plasma concentration tended to decrease. Hypothalamic ir-TRH and its plasma concentration may be expressed as a balance between TRH release, synthesis and degradation. The inactivation of TRH immunoreactivity by plasma or hypothalamus in vitro after eledoisin injection did not differ from that of controls, indicating that eledoisin may affect TRH release or synthesis, but further investigations are needed to clarify this point. Plasma ir-TRH and TSH responses to cold, which are known to be mediated by TRH (Szabo & Frohman 1977), were inhibited by eledoisin. Thus, the present results taken together suggest that eledoisin acts on the hypothalamus to inhibit TRH release.

The release of hypothalamic hormone is reported to be at least partially regulated by amines of the central nervous system (Krulich 1979). Moreover, it has been reported that intracerebroventricular injection of eledoisin produces a long-lasting rise in blood pressure, which is prevented by phenolamine pretreatment (Lambert & Lang 1970). Therefore, a study of eledoisin with amines of the central nervous system would presumably serve to clarify the mechanism of its effect on TSH release. It is well known that L-dopa is a precursor of dopamine, pimozide is a dopamine antagonist, 5-HTP is a precursor of serotonin and PCPA is an inhibitor of serotonin synthesis. The authors used these substances for manipulation of amine levels in the central nervous system.

The present study indicates that the inhibitory effect of eledoisin on TSH levels was prevented in the pimozide-pretreated group, but not in the L-dopa pretreated group. The hypothalamic ir-TRH content tended to increase after L-dopa injection and tended to decrease after pimozide injection, but not significantly. It has been reported that dopamine reduces plasma TSH levels in rats (Krulich 1982). Thus, the present findings suggest that the effects of eledoisin on TSH levels may result from interaction of eledoisin with a dopaminergic system. The inhibitory effect of eledoisin on TSH levels was prevented in the PCPA-pretreated group, but not in the 5-HTP-pretreated group. The hypothalamic ir-TRH content tended to increase after 5-HTP injection, but not significantly. The influence of the serotoninergic system on TSH release has been studied, but results are controversial; Chen & Meites (1975) reported that serotonin stimulated TSH release, but Krulich (1979) and Mitusma & Nogimori (1983) stated that serotonin inhibited it. No changes in plasma TSH levels were observed with 10 mg/kg of 5-HTP or 200 mg/kg of PCPA. The exact reason for this difference is unclear. We have reported that 30 mg/kg of 5-HTP reduced plasma TSH levels (Mitusma & Nogimori 1983). Thus, these differences may be attributed to the experimental conditions such as 5-HTP and PCPA dosage. Therefore, our findings suggest that the effect of eledoisin on TSH levels may result from interaction with a serotoninergic system. The plasma TSH response to TRH did not differ from that of controls, indicating that eledoisin may not act on the pituitary gland. Plasma thyroid hormone levels did not change significantly after eledoisin injection, suggesting that eledoisin may not act on the thyroid gland. However, thyroid hormone metabolism is slower than that of TRH and TSH. Thus, a longer observation period is necessary to clarify this point.

These findings suggest that eledoisin acts on the hypothalamus to inhibit TRH release, and that its effects are at least partially modified by amines of the central nervous system.

Discussion
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References


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