Some other genera also show rather large ranges of chromosome numbers among sibling species, for example, *Equus* $2n = 32$ to 66, *Microtus* $2n = 17$ to 62, *Gerbillus* $2n = 30$ to 66, and *Sigmodon* $2n = 22$ to 52 (*19*). Sibling rodent species of the tribe Ellobini, subfamily Microtinae also vary; *Ellobius talpinus* has $2n = 52$ to 54 and *E. lutescens* has $2n = 17$ in both sexes (*20*).

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## Thyrotropin Secretion in Rats after Hypothalamic Electrical Stimulation or Injection of Synthetic TSH-Releasing Factor

Abstract. *Plasma thyroid-stimulating hormone (TSH) levels, as measured by radioimmunoassay, begin to rise within 5 minutes after initiation of electrical stimulation of the medial-basal hypothalamus, become significantly elevated at 10 minutes, and reach a peak at 10 to 25 minutes. Intravenous administration of synthetic thyrotropin-releasing factor induces a marked rise in plasma thyroid-stimulating hormone which is maximal within 5 minutes after administration. These data are interpreted to indicate that there are neuronal structures within the medial-basal hypothalamus which release preformed thyrotropin-releasing factor. The claim (based on bioassay data) that pyroglutamyl-histidyl-proline amide is a potent thyrotropin-releasing factor has also been confirmed by using a highly specific immunoassay system.*

A number of authors have claimed that electrical stimulation of the hypothalamus causes an increase in pituitary thyroid-stimulating hormone (TSH) secretion. Most of these studies have measured changes in thyroid function as an index of TSH release (*1*). Certain of these experiments suffer from the criticism that non-TSH changes such as vasopressin release (in the rabbit), epinephrine release (in the dog), or other unknown factors such as vascular changes due to sympathetic stimulation might have accounted for the observed results. Increases in plasma TSH as measured by bioassay have been reported in the rat and the rabbit following hypothalamic stimulation (*2*, *3*). The studies in the rat used repeated daily stimulations. Elevation of plasma TSH was found several hours after the last stimulation, but no increases could be detected 1 to 2 hours after a single stimulation of 20 minutes' duration (*2*). In the experiments performed in the rabbit, plasma TSH levels were shown to be increased within 15 minutes after the onset of stimulation, with peak responses occurring in some animals between 15 and 45 minutes. This is the only report of the time course of plasma TSH response after hypothalamic stimulation.

The development of a sensitive and specific radioimmunoassay for TSH in the rat (*4*) has provided the methodology for determination of TSH in small samples of plasma, permitting repeated sampling from the same animal. With this method we have made detailed time-course studies of the effect of electrical stimulation of the hypothalamus. Since it has been hypothesized that the regulation of TSH release is dependent upon the secretion of a hypothalamic "releasing factor" (thyrotropin-releasing factor, TRF), changes in plasma TSH following electrical stimulation were compared with those obtained following the intravenous administration of "synthetic" TRF. Recently two laboratories have reported that a tripeptide amide, pyroglutamyl-histidyl-proline-amide, has thyrotropin-releasing activity identical with that of TRF isolated from hypothalamic extracts and is virtually indistinguishable chemically from the isolated native material (*5*, *6*).

Male Sprague-Dawley rats weighing 250 to 450 g were used. Plasma samples were obtained under pentobarbital (50 mg/kg) or ether anesthesia either by puncture of the external jugular vein or from indwelling jugular cannulas. For electrical stimulation, bipolar .032 gauge Nichrome electrodes, insulated with Insl-X except at the tip, were placed stereotactically into various areas of the medial-basal hypothalamus. The electrodes were soldered to a connector which was affixed to the skull with screws and dental cement. Electrical stimulations were performed 10 to 14 days later. Biphasic square waves from a constant-current stimulator were delivered for 5 or 10 minutes in trains of 4 seconds on and 4 to 10 seconds off, with current of 0.5 to 1.0 ma, frequency of 60 cycle/second, and pulse duration of 1 to 2 msec.

Two separate experiments were undertaken. In the first, electrodes were placed in various regions of the basal hypothalamus extending from the anterior hypothalamus to the posterior ventromedial nucleus. Plasma samples were taken prior to and at several intervals for a period of 1 to 2 hours after stimulation. In the second experiment, electrodes were implanted in the median eminence [de Groot coordinate (*7*): anterior (+)5.6, lateral 0.5, depth (−)3.0], and samples were taken at 0, 5, 10, 15, and 25 minutes.

Two kinds of controls were used. Five sham-stimulated animals with electrodes in place were anesthetized, and blood samples were withdrawn

Table 1. Plasma thyrotropin response to electrical stimulation or "synthetic" TRF administration. Values are milliunits of plasma TSH per 100 milliliters, ± standard error.

| Experiment | Time (min) | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 25 |
| Hypothalamic stimulation | 8.6 ± 3.2 | 14.9 ± 3.2 | 20.6 ± 3.8 | 23.3 ± 8.9 | 20.9 ± 9.4 |
| Cortex stimulation | 11.3 ± 2.0 | 10.5 ± 2.1 | 9.3 ± 2.7 | 6.8 ± 1.0 | 10.6 ± 3.7 |
| 100 ng TRF intravenously | 9.5 ± 2.0 | 97.0 ± 13.3 | 74.5 ± 15.1 | 59.4 ± 11.7 | |
| 0.9 percent NaCl intravenously | 10.6 ± 3.2 | 12.0 ± 4.0 | 5.4 ± 0.4 | 6.6 ± 1.6 | |

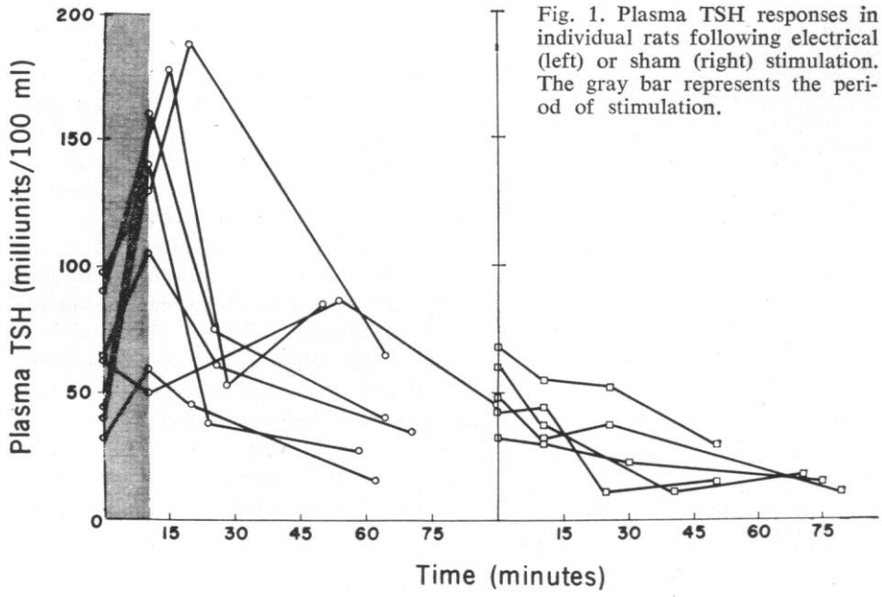


Fig. 1. Plasma TSH responses in individual rats following electrical (left) or sham (right) stimulation. The gray bar represents the period of stimulation.

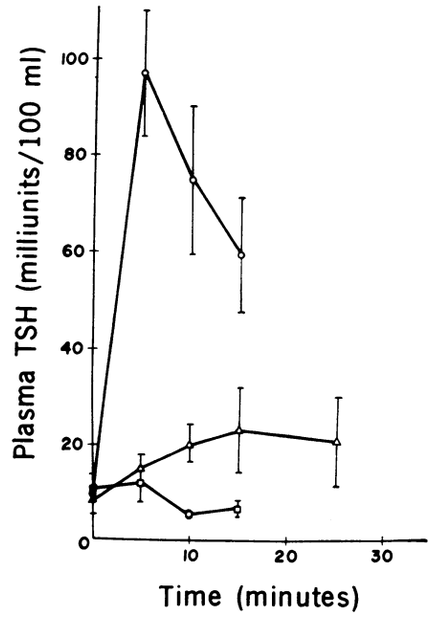


Fig. 2. Comparison of the plasma TSH response following electrical stimulation with that following intravenous administration of "synthetic" TRF. Each point represents the mean of five animals in the experimental groups and four animals in the control group. Circles, "synthetic" TRF; triangles, electrical stimulation; squares, controls; vertical bars, standard errors of the means.

without application of current. Five other animals were stimulated with identical currents through electrodes placed 1 mm below the surface of the parietal cortex. Histological verification of electrode sites was obtained by serial sections of paraffin-embedded brains stained with Luxol fast blue and cresyl violet. There was no evidence of lesions at the site of stimulation. Further proof that the effects were not due to tissue damage was the finding that repeated stimulations at intervals of 2 to 4 weeks in the same animal gave comparable TSH responses.

The amide of the synthetic tripeptide pyroglutamyl-histidyl-proline-acetate (furnished through the courtesy of Hoffman-LaRoche Laboratories) was prepared in our laboratory according to the method described by Burgus *et al.* (*5*). Proof that the product obtained was similar to the material synthesized by Bøler and co-workers (*6*) was shown by thin-layer chromatography of the reaction products in three different solvent systems. One hundred nanograms of the amide were injected into the jugular vein and blood samples were taken for TSH assay at 5-, 10-, and 15-minute intervals. Control rats received normal saline which was used as the diluent for the amide.

Electrical stimulation of a number of sites in the medial-basal hypothalamus resulted in rapid, marked increases in plasma TSH levels, which reached a peak at 10 to 25 minutes after the onset of stimulation (Fig. 1). Positive responses were obtained in seven of eight animals. The electrode placements were all within 1 mm of the midline and no more than 1.5 mm from the base of the hypothalamus; locations extended from the posterior portion of the anterior hypothalamic region to the posterior arcuate nucleus. In the animal in which no response occurred (not shown in the figure), the electrode was located slightly dorsolateral to the ventromedial nucleus, a location which did give a positive response in another animal. One animal showed a later response than the others, a result which may have been in part due to the sampling sequence, since the peak response may have been missed. In the sham-stimulated animals plasma TSH continued to fall during the period of anesthesia, an effect on plasma TSH which has been consistently found in our laboratory with pentobarbital or ether anesthesia.

Since consistent responses appeared to occur with median eminence placements, the time course of TSH release was determined by frequent sampling in animals with electrodes in this region. Stimulation was applied for 5 minutes. The results are shown in Table 1 and Fig. 2. An increase in plasma TSH occurred in all of the five animals stimulated, but the increases were much less than that following the 10-minute stimulations used in the first experiment. An increase in plasma TSH was evident at 5 minutes, but the increase was not significant until 10 minutes ($P < .05$, Student's $t$-test). The peak response appeared to occur at 15 minutes. Stimulation of the cerebral cortex had no effect on plasma TSH levels.

The administration of 100 ng of "synthetic" TRF produced a much more rapid and dramatic response similar in magnitude to the response following 10 minutes of electrical stimulation in the first experiment (Table 1 and Fig. 2). The response was already maximal in the samples taken at 5

minutes and reached levels comparable to those obtained in our laboratory following thyroidectomy. The time course of this response is similar to that which is observed following administration of hypothalamic extracts with TSH-releasing activity (unpublished results).

Control injections of 0.9 percent NaCl, crude extracts of porcine cerebral cortex (equivalent in weight to five stalk median eminences), synthetic mast cell releaser 48/80 (0.03 to 0.5 mg), histamine phosphate (0.25 mg), and Pitressin (0.5 unit) were all without effect on plasma TSH levels.

These experiments demonstrate for the first time the time course of TSH release in the rat following hypothalamic electrical stimulation. The increases in plasma TSH levels were rapid and corresponded closely to those reported for growth hormone following stimulation of the ventromedial hypothalamic nucleus in the rat (*8*). Since peripheral blood was sampled in these experiments, the quantity of TSH secreted must be considerable in order to elevate blood levels two- to fivefold. It is hypothesized that release of TSH from the pituitary is dependent upon a thyrotropin-releasing factor synthesized in the neurons of the basal hypothalamus. The rapid responses which occur following electrical excitation of this region are suggestive of release of preformed TRF from these cells. The administration of "synthetic" TRF in the present experiments produced a more rapid increase in plasma TSH levels, which reached thyroidectomy levels (perhaps a maximal response) within 5 minutes. It is probable that the earlier response following a single injection of TRF reflects the difference between a sudden stimulus and a more sustained release of smaller quantities of TRF during the period of hypothalamic stimulation.

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## Suppression and Elimination of an Island Population of Culex pipiens quinquefasciatus with Sterile Males

Abstract. *The release of 8,400 to 18,000 males per day of* Culex pipiens quinquefasciatus *Say which had been exposed to a sterilizing agent (thiotepa), suppressed and eliminated an indigenous population of this mosquito on an island off the coast of Florida in a 10-week period. Sterile males were effective in seeking out and mating with females on the island.*

*Culex pipiens quinquefasciatus* Say (=*Culex fatigans* Wiedemann) is a vector of human diseases, particularly filariasis, and can be controlled with insecticides. We now report that the technique of male sterilization can be used to suppress population build-ups of *C. p. quinquefasciatus* or to eliminate it from certain areas. We studied methods of sterilization and mass rearing and the biology of this species. In 1968 we released males, sterilized chemically with tepa, on Seahorse Key, located about 3.2 km off the west coast of Florida. These males were competitive with the wild males, and, when the test was discontinued after 8 weeks because of the onset of cool weather, 85 percent of the egg rafts collected were sterile (*1*).

We conducted a similar study in 1969 to show that sterile males could be used to suppress or eliminate an isolated population of *C. p. quinquefasciatus.* The test site was Seahorse Key, a crescent-shaped island, about 1.6 by 0.2 km; it is covered by dense woods except for a large T-shaped clearing that extends across the center of the island.

The primary larval breeding site for *C. p. quinquefasciatus* was a partially open septic tank that received domestic wastes. Breeding also occurred in containers that had been discarded in the woods where they collected rainwater.

The mosquitoes were reared as larvae at the Insects Affecting Man and Animals Research Laboratory at Gainesville, Florida. We sterilized the mosquitoes in the pupal stage by exposing them for 4 hours to an aqueous solution of 0.75 percent thiotepa [tris(1-aziridinyl)phosphine sulfide]. The mosquitoes were then placed on wet blotting paper in pans in Styrofoam boxes chilled with ice and transported to the release site. At Seahorse Key, the pans were placed under the laboratory, the lids were removed, and fresh water was added to float the remaining pupae. The sterile adults dispersed rapidly into the environment, and large numbers were seen in mating swarms at dusk and dawn throughout the cleared area of the island.

Egg rafts were collected from the indigenous population in 12 ovitraps (40-liter tubs containing larval food) located at the edge of the woods and in open areas near the main breeding sites. The egg rafts were collected and counted daily and then placed in separate 18.5-ml vials containing 5 ml of water. Most fertile eggs hatched within 24 hours, but all eggs were kept for 4 days before they were determined to be viable or nonviable. Since the larvae from fertile egg rafts were destroyed, our assay procedures exerted an additional control on the mosquito population similar to what could be achieved with larvicides or removal of breeding sites.

Because fluctuations in egg production caused by varying weather conditions made the daily records difficult to interpret, we combined the data for each