

Differential Effects of Stress on Release of Thyroid-Stimulating Hormone in Young and Old Male Rats^{1, 2} (40009)

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Age-related decreases in the responsiveness of the hypothalamo-hypophyseal system to several kinds of stimuli have been reported in the rat. Old female rats release less luteinizing hormone (LH) and follicle-stimulating hormone (FSH) than young female rats in response to ovariectomy (1, 2) and to progesterone treatment of estrogen-primed ovariectomized rats (3). Similarly, castration and ether stress result in release of less LH and FSH in old than in young male rats (2, 4). In response to acute cold exposure, old male rats release less thyroid-stimulating hormone (TSH) than young males (Huang, Chen and Meites, unpublished). Since all the previously employed stimuli resulted in increased release of anterior pituitary hormones, it was of interest to determine if a centrally mediated inhibition of TSH release changes with age. Acute stress has been shown to result in a rapid decrease in serum TSH in rats (5). It was the purpose of this study to compare the effect of acute stress on TSH release in young and old male rats.

Materials and methods. Male Sprague-Dawley rats were purchased from Charles River, Madison, Wis. at 7 months of age and maintained in our animal quarters until 18-20 months old. For comparison,

2- to 3-month-old male Sprague-Dawley rats were maintained in our laboratory for 1 month before experimentation. Animals were housed in a temperature ($25 \pm 1^\circ$) and light (14 hr daily)-controlled room and were given Purina Rat Chow and water *ad libitum*.

In Experiment I, serial blood samples (1 ml) were obtained by orbital sinus puncture under light ether anesthesia at 0, 2, 6, 12, and 24 hr after an initial bleeding. In Experiment II, animals were stressed by ether exposure for 15 min. Blood samples were taken 1, 15, 30, and 60 min after the initial ether exposure. In Experiment III, physical restraint was accomplished by taping animals to test tube racks for 5 min. Blood samples were taken under light ether anesthesia before and 5, 30, and 60 min after onset of restraint stress. Serum samples from all experiments were stored at -20° until assayed for TSH by the double radioimmunoassay method described in the NIAMDD kit. Serum TSH values are expressed in terms of NIAMDD-TSH-RP-1. Serum thyroxine (T_4) was assayed by the Nichols Institute kit, and values are expressed as micrograms of T_4 per 100 ml of serum in terms of the T_4 standard provided. The significance of differences between bleedings within either age group was determined by analysis of variance and Student-Newman-Keuls test (6). Differences between old and young rats at any time were determined by Student's *t* test. The level of significance chosen was $P < 0.05$.

Results. In Experiment I (Table 1), serial bleeding in young males resulted in a 67% decrease in serum TSH at 6 hr, an 80% decrease at 12 hr, and a 48% reduction below zero time at 24 hr. In old males serial bleeding resulted in no change in serum TSH at 6 hr, a 33% decrease at 12

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hr, and a 31% reduction at 24 hr. Serum T_4 was initially lower in old than in young males. Serum T_4 in young males followed a pattern similar to serum TSH, being depressed at 6, 12, and 24 hr as compared with the initial value. In old males, serum T_4 levels were correlated with TSH levels except at 2 hr when serum TSH was high and serum T_4 was low.

Acute ether exposure in young males (Fig. 1) resulted in a significant decrease in serum TSH at 30 and 60 min after initial ether exposure. No change in serum TSH occurred in old males during the same time period. In response to 5 min of physical restraint (Fig. 2), serum TSH in young males decreased 65% by 5 min after the onset of restraint and remained low through 60 min. In old males, physical restraint had no significant effect on serum TSH, although serum levels tended to decrease with time.

Discussion. These results demonstrate that, in response to several kinds of stress, serum TSH decreases more rapidly and to a greater extent in young than in old male rats. Since acute stress is known to inhibit TSH secretion in rats (5, 7, 8), it appears that the central mechanism that mediates TSH inhibition is less sensitive in old than in young male rats. These findings are consistent with the observation of an age-related decrease in ability of the hypothalamus to inhibit prolactin secretion tonically (9, 10). The inability of old males to exhibit a normal TSH response to stress is similar to the report of Riegler and Meites (4) that stress-induced LH release does not occur in old male rats. In contrast, there appears to be little age-related

change in secretion of adenocorticotropin (ACTH) in response to chronic stress (11).

The degree of TSH inhibition in young

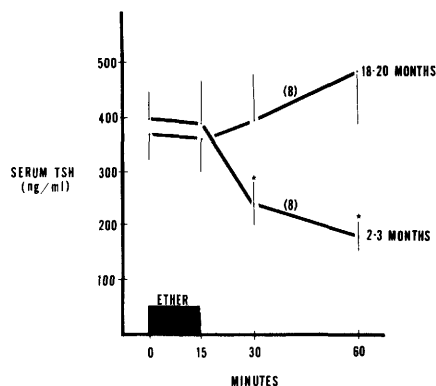


Fig. 1. Effect of 15 min of ether exposure on serum TSH in young and old male rats. Star indicates significant difference from initial sample. Numbers within parentheses indicate number of animals per determination. Solid bar indicates time of ether exposure.

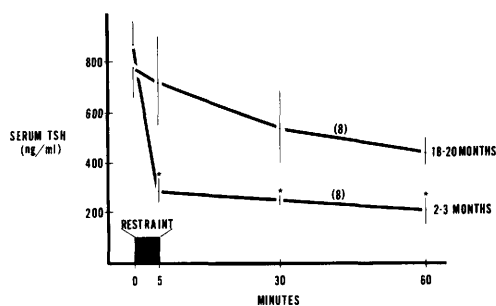


Fig. 2. Effect of 5 min of restraint stress on serum TSH in young and old male rats. Star indicates significant difference from initial sample. Numbers within parentheses indicate number of animals per determination. Solid bar indicates time of restraint.

TABLE I. EFFECT OF SERIAL BLEEDING ON SERUM TSH AND T_4 IN YOUNG AND OLD MALE RATS

Age ^a	Hours after initial bleeding				
	0	2	6	12	24
Serum TSH (ng/ml)					
3-4 months	994 ± 159	775 ± 123	329 ± 68 ^b	198 ± 55 ^b	513 ± 68 ^b
18 months	651 ± 70	790 ± 61	621 ± 75	433 ± 75 ^b	448 ± 49 ^b
Serum T_4 (μ g/100 ml)					
3-4 months	5.2 ± 0.2	4.9 ± 0.3	2.9 ± 0.3 ^b	3.1 ± 0.2 ^b	2.6 ± 0.2 ^b
18 months	4.2 ± 0.3	3.3 ± 0.3 ^b	4.7 ± 0.7	2.4 ± 0.3 ^b	2.8 ± 0.2 ^b

^a Twelve or thirteen animals per determination.

^b Difference from initial sample is significant ($P < 0.05$).

males was less in response to ether than to restraint stress. Acute ether exposure resulted in a 39% decrease in serum TSH by 30 min, which is similar to that reported by Fukuda *et al.* (12) and Männistö *et al.* (13). In contrast, physical restraint in young males resulted in a 65% decrease in serum TSH by 5 min. Mueller *et al.* (8) reported a 46% decrease in serum TSH after 5 min of restraint stress in rats killed by decapitation. The animals subjected to restraint stress were bled under light ether anesthesia. Thus the magnitude of restraint-induced TSH inhibition probably reflects the sum of both ether and physical restraint and could account for the larger decrease in serum TSH observed in the present study. In old males, physical restraint was also more effective than ether in decreasing TSH, although both stresses were less effective in old than in young rats.

Basal levels of serum TSH in old males tended to be lower than TSH levels in young males in this and in a previous report (10). The large variance in basal levels of serum TSH in both old and young males prevented these differences from being significant. Since serum T_4 also was lower in old than in young males, the age-related trend toward a decrease in basal levels of serum TSH appears to result from changes at the hypothalamo-hypophyseal level rather than to inhibitory feedback from T_4 .

Since stresses have been reported to increase the turnover of catecholamines (14-16) and indoleamines (8, 17-19) in the hypothalamus of rats, the possibility exists that alterations in the ability of the autonomic nervous system to mediate a stress response occur with aging. Recently, we reported evidence for a decrease in DA turnover in the medial basal hypothalamus of old male rats (9), and there is considerable evidence that DA can inhibit TSH release. Thus the DA agonists, apomorphine and piribedil, have been reported to decrease basal serum TSH levels in male rats (20), and apomorphine blocked cold-induced TSH release (21). Therefore the decreased DA turnover in old male rats may be partially responsible

for the lack of inhibition of TSH in response to stress.

Restraint and electrical shock also were reported to increase serotonergic activity in several brain regions, including the hypothalamus, of young rats (8, 17, 18). In old male rats, hypothalamic serotonin turnover appears to be elevated (10). Since serotonin influences on TSH release are in dispute (8, 22-25), it is difficult to assess its possible effects on TSH secretion during stress. However, restraint stress has been observed to increase hypothalamic serotonin turnover and at the same time to depress serum TSH levels in male rats (8). However, the relation of serotonin to the results observed here on TSH release in old male rats cannot be explained at present.

Glucocorticoids have been shown to inhibit the secretion of TSH (7) and may have a role in the stress-induced inhibition of TSH secretion in old male rats. Riegler (11) reported a slight decrease in the ability of adrenals to secrete corticosterone in response to ACTH in old male rats. This apparent decrease in sensitivity of the adrenals of old male rats could account in part for the absence of the stress-induced TSH decrease. Further work is necessary to determine which of these mechanisms is responsible for the decline in capacity of the hypothalamo-hypophyseal system of old male rats to regulate TSH secretion in response to stress.

Summary. In response to the stress of serial bleeding, ether exposure, or physical restraint, serum TSH was depressed more quickly and to a greater extent in young than in old male rats. These results suggest that the hypothalamo-hypophyseal mechanisms which mediate inhibition of TSH release are less sensitive in old than in young male rats.

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