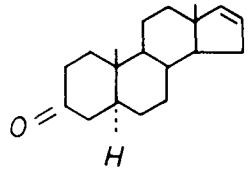
She was familiar, from her husbands work, with the characteristic smell of boar taint, and noticed this smell when cooking parsnips grown in her garden. Therefore analytical screening in parsnip was carried out by introducing extracts from the root into the routine radioimmunoassay (RIA) for 5a-androst-16-en-3-one[4]. The 1st extracts assayed gave positive results. Consequently further analytical investigations in various vegetables were carried out: 100 g of vegetable were homogenized with 250 ml of water. Aliquot portions (0.5–2.0 ml) were removed, extracted with methylene chloride and the evaporated extract was directly measured radioimmunologically. The main part of the homogenate was mixed with 200,000 dpm ^{3}H-5a-androst-16-en-3-one (sp. act. 15 Ci/mM), extracted 2 times with methylene chloride/ethyl acetate = 1/1 (v/v) and the solvent was evaporated.

Extracts were purified on silica gel columns (270 × 10 mm) using cyclohexane/ethyl acetate = 98/2 (v/v), further on thin-layer plates (silica gel; Merck, Darmstadt) by the system benzene/acetone = 90/10. Aliquot parts of the eluate were measured radioimmunologically. Thus parsnip samples were measured from the 1977- and 1978-harvest and from the market. For comparison, carrots (*Daucus carota* L.), potatoes (*Solanum tuberosum*), fennel (*Foeniculum vulgare*), radish (*Raphanus sativus*), scorsonera (*Scorzonera hispanica*), parsley (*Petroselinum hortense*) and – due to some similarities to the taste of parsnip – celery were treated and measured identically. Results of the radioimmunological measurements in crude and purified extracts of parsnip roots are shown in the table.



5a-androst-16-en-3-one ('boar taint')

5a-androst-16-en-3-one ('boar taint steroid') in roots of the parsnip plant (concentrations in ng/g root; measurements after purification corrected for procedural losses)

Grown 1977		Grown 1978		From the market	
Crude extract	Purified extract	Crude extract	Purified extract	Crude extract	Purified extract
6.3	5.5	11.4	9.6	9.5	8.0

In all 3 pools of samples, the presence of 5a-androst-16-en-3-one could be clearly demonstrated by RIA. Concentrations in crude extracts are only slightly higher compared to extracts where the steroid was isolated by chromatography. In crude extract from celery, a concentration of 9 ng/g plant was detectable, in purified extracts 5.6 ng/g plant were measured.

Surprisingly, the concentrations are remarkably high. For comparison, concentrations in peripheral blood-plasma of mature boars (expressed in ng/ml plasma) are in the same range. In the other vegetables, measurements revealed negative results.

The identity in parsnip as well as in celery was confirmed by combined gas chromatography – mass spectrometry (GLC-MS; LKB 2091 instrument, 1.5 m GLC glass column with 1% OV 3 on chromosorb WHP operated at 195 °C; 20 ml He/min). GLC retention time (5.8 min) as well as molecular weight (m/e 272) and the fragmentation pattern of the substance from parsnip and celery extracts were identical with authentic 5a-androst-16-en-3-one[2].

The occurrence of steroidal compounds in plants is not unique. Oestrogens, for example, were isolated from plants as early as 1933[4]. It is also known that some plants may mimic pheromonal substances[5]. At the moment, biological function – if any – of boar taint substance in parsnip is not known. Neither is it known if the boar taint substance in celery contributes to the 'libido-supporting' property for which this plant has some popularity.

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Control of the hypothalamus-pituitary-adrenal axis in young and older rats injected with thyroxine[1-3]

L.A. Meserve

Department of Biological Sciences, Bowling Green State University, Bowling Green (Ohio 43403, USA), 9 March 1979

Summary. Administration of T_4 on alternate weeks for 30 weeks at a dosage which does not alter body weight depresses basal serum corticosterone levels in older rats (575 days), but not in young animals (260 days). Similar serum corticosterone response to HPA axis stimulation occurs regardless of age or T_4 injection.

The depression of activity of the hypothalamus-pituitary-adrenal (HPA) axis with age has been reported in numerous species from mice[4] to men[5], including rats[6]. On the other hand, some investigators have reported similar HPA response 15 min after stress in 2.5-, 7.5-, 12-, 18-and 26-month-old rats[7]. Studies of other aging neuroendocrine axes have included demonstration of altered control of hypothalamus-pituitary-thyroid system, resulting in depressed circulating thyroid hormone levels[8-10]. Since hypothyroidism alters the response of the HPA axis to stress in the rat[11] and mouse[12], the present study was conducted to determine the influence of low doses of thyroxine on HPA function in young and older rats.

Materials and methods. Male Holtzman rats were obtained from the supplier and started on experiment at either 50 days or approximately 1 year (retired breeders) of age. L-thyroxine (T_4, sodium salt) was injected s.c. 6 days per week, every other week for 30 weeks, according to the schedule of Everitt[13] based on the data of Grad and Hoffman[14] for a low hormone dosage. Young rats received a total of 0.825 mg T_4 in 90 injections and older rats received 1.65 mg on the same schedule. Control animals were administered saline injection vehicle. At the end of the 30th week, 44 animals per age and thyroid status group were randomly divided into 4 HPA test groups of 11 animals each. HPA response was measured by comparing the adrenal and serum corticosterone (B) concentrations in unstimulated rats (basal) to those in animals 15 min after either: exposure to ether fumes for 1 min (ether stress); s.c. injection of 8 IU depot adrenocorticotropin[15] (exogenous

Table 1. Body weights and relative pituitary, thyroid, and adrenal gland weights in young and older rats after saline or thyroxine injection

Group	Body weight (g)	Pituitary (mg/100 g b.wt)	Thyroid (mg/100 g b.wt)	Adrenals (mg/100 g b.wt)
Young saline	584.8 ± 7.6[a]	3.0 ± 0.1	5.2 ± 0.2	4.9 ± 0.2
Young thyroxine	580.4 ± 9.5	2.3 ± 0.1[b]	4.4 ± 0.1[b]	5.1 ± 0.2
Older saline	650.4 ± 10.9	2.4 ± 0.1[b]	4.5 ± 0.1[b]	4.5 ± 0.2
Older thyroxine	651.1 ± 10.5	2.4 ± 0.1[b]	4.4 ± 0.4	4.1 ± 0.1[b]

[a] Mean ± SEM for 44 animals per group; [b] Significantly different from young saline ($p < 0.01$).

Table 2. Adrenal and serum corticosterone (B) concentrations in stimulated or unstimulated young and older rats injected with saline or thyroxine

Group	Basal	Ether[a]	ACTH[b]	Saline[c]
Adrenal B (μg/100 mg)				
Young saline	2.50 ± 0.08[d]	3.79 ± 0.08[e]	3.75 ± 0.10[e]	3.61 ± 0.10[e]
Young thyroxine	1.90 ± 0.06	4.16 ± 0.12[e]	3.91 ± 0.10[e]	4.34 ± 0.10[e]
Older saline	2.30 ± 0.11	3.87 ± 0.14[e]	4.27 ± 0.21[e]	3.53 ± 0.18[e]
Older thyroxine	2.40 ± 0.07	4.46 ± 0.24[e]	4.32 ± 0.10[e]	3.53 ± 0.10[e]
Serum B (μg/100 ml)				
Young saline	7.88 ± 0.42	30.53 ± 0.85[e]	28.73 ± 1.05[e]	23.50 ± 0.64[e]
Young thyroxine	7.30 ± 0.28	30.33 ± 0.62[e]	29.34 ± 0.95[e]	22.57 ± 1.10[e]
Older saline	8.77 ± 0.56	29.40 ± 0.62[e]	28.91 ± 0.93[e]	28.66 ± 0.95[e]
Older thyroxine	5.83 ± 0.33[f]	26.90 ± 0.91[e]	29.80 ± 0.62[e]	24.13 ± 0.81[e]

[a] Tested 15 min after initiation of a 1 min ether stress. [b] Tested 15 min after a s.c. depot injection of 8IU ACTH. [c] Tested 15 min after a s.c. injection of physiological saline. [d] Mean ± SEM for 11 rats per group. [e] Greater than basal levels for the same age and thyroid status ($p < 0.01$). [f] Different from basal levels of older saline rats ($p < 0.05$).

ACTH); or s.c. injection of saline (ACTH injection placebo). Adrenal and serum B concentrations were determined by the fluorometric method of Hilf et al.[16] with minor modifications. Data were statistically analyzed by an analysis of variance and multiple comparisons using log transformation where appropriate. $p < 0.05$ was considered significant.

Results. Body weight at the end of 30 weeks was not altered by the dosages of T_4 administered to either young or older rats. Young animals showed the expected decrease in relative weights of pituitary and thyroid glands after T_4 injection, but relative adrenal gland weight was not significantly changed (table 1). On the other hand, the relative weights of pituitary and thyroid glands in older rats remained constant after T_4 administration, suggesting subnormal sensitivity to circulating thyroid hormone levels. Stimulation of the HPA axis by any of the manipulations used (ether stress, ACTH injection, saline injection) increased adrenal B concentration an average of 74% regardless of age or thyroid status (table 2). Observation of serum B revealed a 200–300% increase after stimulation in both control and T_4 injected young rats. Although the post-stimulus serum B levels of older rats did not differ significantly from those in young animals, T_4 significantly depressed basal serum B levels in the older rats (table 2). Consequently, the stimulus induced serum B increase was approximately 230% in control and 360% in T_4 injected older rats.

Discussion. The present data confirm the results of Tang and Phillips[7], showing the HPA axis of young (7.5 or 9 months) and older (18 or 19 months) animals to respond similarly to acute stress. Although methods of B assay were different (competitive protein binding vs fluorometric), reported circulating steroid concentrations appear to be similar in the 2 studies. Tang and Phillips[7] suggest an age related increase in basal circulating B levels (2.2 vs 3.5 μg/100 ml) as does the present study (7.9 vs 8.8 μg/100 ml). However, the difference in each case is not significant. The possible basal serum B increase may be the result of depressed HPA feedback control in aged rats as reported by Hess and Riegle[17]. Tang and Phillips[7] support this finding by demonstrating a constant increase in basal levels of circulating ACTH with age. The present study gives indirect evidence that T_4 injection improves feedback sensitivity of the HPA axis, since older rats treated with T_4 carry lower concentrations of serum B than control animals. Measurement of circulating ACTH levels after T_4 administration would add more information about the feedback sensitivity of the axis.

To more fully determine the influence of thyroid hormone on HPA axis control in aging rats, it will be necessary to study a wider range of ages, to treat with varying doses of T_4, to determine the effect of triiodothyronine (T_3) injection, and to investigate the response to chronic as well as acute stress.

1 Presented in part at the 8th Annual National Meeting of the American Aging Association, October 1978, San Francisco.
2 Supported by an FRC grant, Graduate College, Bowling Green State University.
3 Acknowledgment is made of the technical assistance of L. Carter, P. Froelich, R. Froelich, J. Ku, M. Meserve and D. Schulte.
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