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Time Study of Acute Cold-Induced Acceleration of Thyroidal I^{131} Release in the Hamster.* (25840)

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Previous experiments(1,2) have shown that rate of hormone release from hamster thyroid is accelerated markedly during first 12 hours exposure to cold (5-6°C). The data indicated that this acute release of thyroidal I^{131} is dependent upon a neural mechanism which discharges TSH from the pituitary gland. The present study is a more careful analysis of some hypothalamo-pituitary-thyroid relationships during this initial 12 hour period of cold.

Methods. Adult hamsters of both sexes weighing 90-110 g were used. Twelve to 18 hours after intraperitoneal injection of 4-8 μc of I^{131}, thyroidal uptake was measured by counting externally neck and hind-leg regions with shielded scintillation crystal. The neck-minus leg counts, expressed as per cent of injected dose, represented thyroid uptake. The fractional rate of thyroidal I^{131} release was estimated by subsequent daily measurement of thyroid activity, each day's calculation being corrected for physical decay and expressed as percentage of initial 12 hour uptake. After at least 3 days of external counting to determine normal slope of release curve and immediately after zero count at room temperature (20-22°C), groups of hamsters were exposed to cold (5-6°) for following periods: 0.5, 1, 2, 3, 4, 6, 8 and 12 hours. During periods of cold exposure, animals were housed individually in metal cages with minimum of bedding; free access to food and water was permitted. After desired period of cold, thyroid activity was again measured externally, blood samples taken for determination of PBI^{127} and thyroid glands removed and weighed. Radioactivity was measured on one excised lobe, then prepared for determination of total iodine content. Posterior lobes of pituitary glands were separated by blunt dissection and adenohypophyses of at least 8 animals of each group were pooled, homogenized in acidified saline and frozen until assayed for thyrotropin (TSH) content. A time-study of response of hamster thyroid to exogenously administered TSH was carried out in same fashion as the time-study of thyroid response to cold. After zero external count, not less than 10 animals/group were injected intraperitoneally with one unit of TSH (Armour Thtropar). Ability of thyroxine to block cold-induced release of thyroidal I^{131} was examined. Freshly dissolved L-thyroxine was administered subcutaneously to groups of not less than 8 animals in doses ranging from 2.5 to 20 μg at varying times before and after beginning of 12 hours of cold exposure.

Results. Response to cold. To express quantitatively the accelerated release produced by varying periods of cold exposure, normal (room temperature) release curves of each animal were continued, appropriate number of hours (corresponding to number of hours of cold) to determine a theoretical position of the curve at room temperature. The difference between this point and the actual position of release curve determined after cold exposure is considered per cent of accelerated release. Fig. 1 summarizes time sequence of accelerated release from hamster thyroid at-

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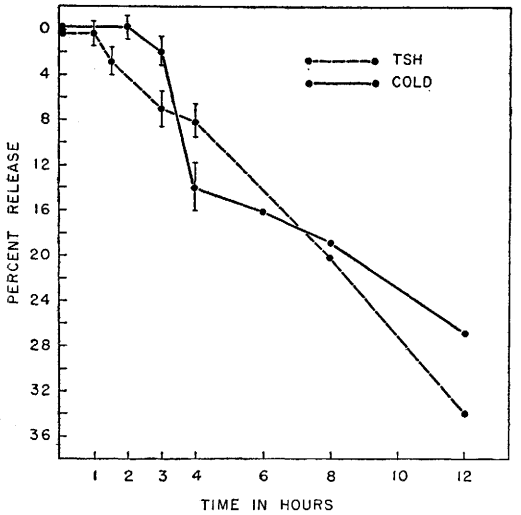

FIG. 1. Thyroidal I^{131} release in the hamster following cold exposure or thyrotropin administration.

tendant upon acute cold exposure. No accelerated release occurs during first 2 hours of cold; after 3 hours a detectable (but not statistically significant) change in rate of release is seen. During 3rd to 4th hour of cold a maximal rate of release occurs. After 4th hour, the rate is slower but continues to fall until a 25-30% release is achieved at 12 hours of cold. Cold-induced disappearance of thyroidal I^{131} from the gland is paralleled by a fall in chemically determined iodine content (Table I). A discrepancy is noted, however, in that after 2 hours of cold no evidence of release based upon thyroidal I^{131} measurements is seen, while total iodine determinations indicate a fall of approximately 18%. Although the discrepancy between these 2 measurements of thyroid response cannot be fully explained, it may represent intraglandular deiodination(3) or a pool of thyroidal organic and inorganic iodide(4) which is not radio-labeled and which responds earlier to administration of TSH. During the critical period of first 4 hours of cold exposure PBI, values were determined on samples of blood from each of 6 control animals and 6 exposed for 2, 3 and 4 hours. PBI level in control hamsters is 2.81 $\pm$ .31 mg %. No significant change in PBI level occurs during 2-4 hours of cold exposure, the respective values being 2.83, 2.78 and 2.83 mg % (Table I).

Response to Thyrotropin. A significant acceleration in thyroidal I^{131} release produced by exogenously administered TSH is first observed 1.5 hours after administration. Rate of accelerated release continues thereafter at approximately the same slope throughout the 12 hour period studied and produces a slightly greater final total per cent release than does 12 hours of cold exposure (Fig. 1).

Pituitary Thyrotropin. TSH content in hamster pituitary was estimated by using the assay of Greenspan *et al.*(5), based upon uptake of P^{32} by chick thyroid. USP thyrotropin standards were used in doses of 0.4 and 4.0 milliunits. Extracts of pooled pituitary glands from several groups of animals were adjusted to administer 0.1 ml intracardially at 2 dose levels estimated to be equal to that of the standards. Seven chicks were used/group with 5 μc P^{32} being administered subcutaneously 3 hours after TSH and the chicks killed 3 hours after radiophosphorus. Thyroids were dissected under a binocular dissecting microscope, dried and their radioactivity measured using a thin window GM tube. Pituitary TSH content of cold-exposed hamsters and corresponding control (room temperature) animals was determined in 3 separate assays for each cold period studied. TSH content after 2 hours of cold was determined in 2 assays.

No significant sex difference in TSH concentration is found between pituitary glands of male and female hamsters, normal concentration being 0.68 $\pm$ .11 milliunit/mg (Table II). A 60% decrease in TSH concentration results after one hour. This decreases further until a concentration of 0.16 milli-

TABLE I. Thyroid Iodine Concentration and PBI after Acute Cold Exposure of the Hamster.

Hr after cold	Thyroid I^{127}, μg/100 mg	PBI, μg %
0	(16) 15.1 $\pm$ 1.1*	(6) 2.81 $\pm$ .31
2	(12) 12.4 $\pm$ .9	(6) 2.83 $\pm$ .22
3		(6) 2.78 $\pm$ .30
4	(8) 11.3 $\pm$ 1.4	(6) 2.83 $\pm$ .18
6	(8) 10.6 $\pm$ 1.3	
8	(8) 10.1 $\pm$ .8	
12	(9) 9.7 $\pm$ .8	

* No. of determinations (in parentheses), mean $\pm$ S.D.

TABLE II. Influence of Cold Exposure upon Concentration of Thyrotropin in Pituitary Glands of Hamsters.

Cold exposure, hr	No. animals	No. assays	Pituitary wt, mg	Pituitary TSH, mu/mg
0	38	6	3.08 ±.04†	.68 ±.11
0.5	18	3	3.44 ±.07	.54 ±.13
1	16	3	3.26 ±.03	.24 ±.06*
2	13	2	3.46 ±.07	.38 ±.08*
4	18	3	3.21 ±.07	.30 ±.10*
7-8	18	3	3.38 ±.10	.17 ±.05*
12	18	3	3.58 ±.06	.16 ±.07*

* Significant difference (P = .001) from control.
† Mean ± S.D.

unit/mg is present at 7-12 hours after cold.

Inhibition of release by thyroxine. Inhibition of cold-induced acceleration was considered complete when thyroidal I^{131} release of less than 10% was observed after 12 hours at 5-6°C. A release of greater than 25% represented no inhibiting effect. Single doses of 2.5 and 5 μg of thyroxine inhibit thyroidal I^{131} release in hamsters maintained at room temperature, but are unable, regardless of when administered, to inhibit accelerated release precipitated by 12 hours of cold exposure. From 2 hours before until 2 hours after cold exposure, 10 μg of thyroxine blocks release consistently. After 4 hours of cold, 10 μg of thyroxine is ineffective while at 3 hours, results are inconsistent with approximately equal numbers of animals exhibiting no block, partial, or complete inhibition of release.

Discussion. Influence of the central nervous system in normal pituitary-thyroid relationships is demonstrated by evidence that electrical destruction of various hypothalamic regions results in disability of pituitary gland in supplying increased amounts of thyrotropin under a variety of experimental conditions (goitrogens, cold exposure, thyroidectomy) which normally evoke pituitary-thyroid responses(6-9). Under proper conditions, acute cold appears to evoke a neural mechanism which discharges pituitary TSH similar to stress-induced release of ACTH. Our experiments permit a more detailed description of neural and hormonal events occurring during initial 12 hours of cold exposure of the hamster. Time-study of thyroidal I^{131} release in response to cold reveals that acceleration begins at or shortly after 3 hours. TSH (exogenous) produces a detectable increase in rate of release 1.5 hours after administration. Time sequence of action of one dose of exogenously administered TSH cannot be compared strictly with the action of endogenous TSH released from the pituitary. However, sudden depletion of a major part (60%) of the pituitary store of TSH during 0.5-1 hour after cold, appears almost comparable to a one-injection phenomenon itself. This is further emphasized by very rapid acceleration in thyroidal I^{131} release observed during third and fourth hours of cold. Intraperitoneally injected TSH, absorbed rapidly but apparently at a more constant rate, produces accelerated release whose slope is more constant throughout the 12 hours studied. Acute cold exposure of rat(10) and rabbit(11) also evoke neural mechanisms for discharge of TSH. In rabbits, increased blood TSH is detectable within 30 minutes of exposure; maximal amounts (500-fold increase) are achieved after 3 hours(12). PBI determinations revealed that no major change in peripheral levels of thyroid hormone were responsible for initiation of TSH discharge and thyroidal I^{131} release. It is possible that pituitary and/or responsive CNS areas are more sensitive to circulating levels of thyroxine than is revealed by this parameter.

The blocking effect of thyroxine bears significantly upon the question of site of action of thyroxine in feed-back control mechanisms operating between thyroid and pituitary. Thyroxine blocked thyroidal release when administered 2-3 hours after cold. Once accelerated, release had begun from the thyroid (at or shortly after the third hour) thyroxine had no effect. Additionally, the fact that the major stimulating surge of pituitary TSH was released within one hour after cold and exogenous thyroxine was then still able to inhibit thyroidal release when administered at 2-3 hours after cold, leads to the possibility that under these conditions of cold exposure of the hamster, thyroxine may be acting at thyroid level to prevent thyroidal release or in some way interfere with the action of TSH

in evoking release. A feed-back effect at pituitary level has been demonstrated by direct micro-injection of thyroxine into the pituitary (13,14). Yamada and Greer(14) and Yamada(15) have, furthermore, demonstrated an inhibitory effect on TSH release by micro-injection of thyroxine into anterior hypothalamus of the rat. Integrity of these hypothalamic areas is, however, not essential for feed-back inhibition since thyroxine is still effective after electrolytic destruction of anterior hypothalamus(16,17). A possible role of other brain areas is suggested by Mess(18-20) who demonstrated that the depressing effect of thyroxine upon thyroid weight and pituitary TSH content was notably modified by lesions of the habenula complex.

Summary. Analysis of time sequence in pituitary and thyroid phenomena involved during acute response of hamsters to cold exposure indicates a rapidly activated neural component. Within 0.5-1 hour after cold, sensory perception of this temperature change has generated sufficient input into hypothalamic effector mechanisms to deplete pituitary TSH by 60%. After a latent period of 1.5 hours, accelerated release begins and results in 25-30% decrease of thyroidal I^{131} during 12 hours of cold. Thyroxine (10 μg) is capable of inhibiting thyroidal I^{131} release when administered as long as 2-3 hours after exposure to cold.

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Cholesterol Concentration in Human Serum and Blood Cells.* (25841)

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The concentration of cholesterol in human erythrocytes has been reported to be much less variable than in plasma and relatively independent of the latter value(1,2). However, the data of Foldes and Murphy(3) from 20 "normals" aged 19 to 35 and 20 surgical patients aged 70 to 90 indicate considerable variation between individuals, the standard deviation of red cell concentration in the normals being 27.6 mg per 100 ml, or 16% of the mean of 173.0. The variability was equally large when the 2 age groups were separately considered. Moreover, when plasma cholesterol changes were induced by treatment of 17 thyroid patients the data reported indicate cell cholesterol concentration changes in the

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