Thyrotropin Releasing Hormone: Enhancement of Dopa Activity by a Hypothalamic Hormone

Abstract. *Thyrotropin releasing hormone potentiates the behaviorial effects of dopa plus pargyline in mice. Because the potentiation occurs in hypophysectomized mice, as well as in normal mice, the phenomenon is independent of the release of thyroid stimulating hormone from the pituitary. Possible mechanisms and clinical implications are discussed.*

After a series of studies (*1*) that illuminated certain aspects of the interaction between imipramine and thyroid state in animals and man, Prange and associates (*2*) reported the therapeutic advantage of combining L-triiodothyronine (T3) and imipramine in the treatment of depression. A later study by these investigators showed that thyroid stimulating hormone (TSH) is also capable of enhancing the therapeutic properties of imipramine (*3*), probably by evoking thyroid hormone secretion. Since the hypothalamic hormone thyrotropin releasing hormone (TRH) acts on the pituitary to release TSH, which in turn prompts thyroid hormone secretion (*4*), it became of interest to discern whether TRH shares properties with T3. Breese and his colleagues (*5*) have shown that T3, with or without imipramine, will potentiate the increased activity of mice after a threshold dose of L-dopa plus pargyline (*6*). We evaluated the action of TRH in the dopa plus pargyline potentiation test in normal as well as hypophysectomized mice.

The dopa plus pargyline potentiation test (*6*) consists essentially of the assessment of the motor response of aggregated mice that received preliminary treatment with a low dose of a monoamine oxidase inhibitor pargyline (40 mg/kg, orally) and then received TRH and a dose of L-dopa (100 mg/kg, intraperitoneally. Normal (intact) male mice (17 to 22 g) (ICR, Madison, Wis.) were treated with pargyline before treatment with dopa. The TRH was administered intraperitoneally or orally 1, 4, 8, or 24 hours prior to dopa. The scoring system described by Everett was used (*6*). Experienced observers, unaware of treatment, rated groups of four mice for 1 hour after the administration of dopa. At each session, it was verified that pargyline plus dopa produced only threshold activity. The same experimental technique was used in hypophysectomized male mice (16 to 18 g) (CD-1, Charles River).

The TRH caused maximum potentiation of dopa plus pargyline when given 1, 4, or 8 hours earlier (Table 1). The TRH was found to be active in the dopa potentiation test by either the oral route or the intraperitoneal route. This would seem to eliminate abdominal irritations by TRH as a possible cause of the results. Only a moderate potentiation was observed when TRH was given 24 hours earlier. Marked behavioral potentiation of dopa plus pargyline was noted at doses of TRH greater than 0.4 mg/kg when the dose of pargyline was 40 mg/kg. In other experiments in which mice were treated with pargyline (50 mg/kg), doses of TRH in excess of 0.1 mg/kg produced a maximum rating (data not shown). When mice received TRH plus pargyline in the above doses and when dopa was omitted, no behavioral effects occurred. Hypophysectomy did not reduce the potentiation of the behavioral effects of dopa plus pargyline by TSH at doses of 0.4 to 0.8 mg/kg (Table 1) and appeared to increase it at lower doses.

Thyrotropin releasing hormone is a hypothalamic releasing hormone and is carried by way of the hypothalamic-hypophyseal portal system to the anterior pituitary where the release of TSH in turn acts on the thyroid gland (*4*). Thyrotropin releasing hormone also causes pituitary release of prolactin (*7*) but does not stimulate release of luteinizing hormone, follicle stimulating hormone, and adrenocorticotropin (*4*). The observation that hypophysectomy did not diminish the reaction to dopa plus pargyline after TRH indicates that the action of TRH is independent of thyroid hormone release by TSH. Therefore, unless TRH is found to release hormone directly from the thyroid gland, a mechanism of action of TRH must be proposed that is independent of thyroid hormone activity.

Another of the hypothalamic releasing hormones, melanocyte stimulating hormone release inhibiting hormone (*8*), has also been found to enhance the behavioral reaction of mice to dopa plus pargyline. Whether these hormones share a common mechanism to produce this effect has yet to be determined. In regard to the action of TRH in producing dopa plus pargyline potentiation, two prominent possibilities would seem to warrant further examination. (i) The TRH may increase receptor sensitivity in dopaminergic neurons. (ii) The TRH may alter the metabolism of dopa in such a manner as to increase the concentration of active dopamine in brain. Studies are currently under way to examine these possibilities.

Apart from the contribution of these studies to a basic understanding of the properties of TRH, they may have clinical importance. The dopa plus pargyline potentiation test was devised to screen drugs for antidepressant activity and has proved useful in this regard. Breese *et al.* (*5*) showed that T3

Table 1. Dopa plus pargyline potentiation by TRH in mice. The TRH was administered 1, 4, 8, or 24 hours prior to dopa. The degree of TRH plus dopa plus pargyline potentiation was: 1, slight; 2, moderate; or 3, marked. At each experimental session control mice showed only slight effects. Control mice received pargyline plus dopa. Three experiments were run in normal mice at 1 hour and two in hypophysectomized mice (intraperitoneal route). Starred entries were not tested.

TRH (mg/kg)	Degree of behavioral potentiation in mice								
	Normal								Hypophysectomized
	1 hour			4 hours	8 hours	24 hours			1 hour
	Intraperitoneal route								
0.05	1	2	1	*	*	*	2	3	
0.1	1	2	1	1	1	1	2	3	
0.2	2	2	2	2	2	1	2	3	
0.4	3	3	3	3	2	1	2	3	
0.8	3	3	3	3	3	1	3	3	
1.6	*	*	*	*	*	2	*	3	
Controls	1	1	1	1	1	1	1	1	
	Oral route								
0.05	1	1	1	*	*	*		*	
0.1	1	1	1	1	*	*		2	
0.2	1	1	1	1	1	1		3	
0.4	2	2	1	3	1	1		3	
0.8	3	3	3	3	1	1		3	
1.6	*	*	*	*	2	1		3	
Controls	1	1	1	1	1	1		1	

amplified the action of imipramine in this test; Prange *et al.* (*2*) had shown that T3 augmented the antidepressant action of imipramine. Further, Breese *et al.* (*5*) showed that T3 alone was active in the dopa plus pargyline test. Subsequently, Prange and Wilson (*9*) found that T3 alone given briefly in moderately large doses was comparable to imipramine in antidepressant activity. This paradigm, taken with our findings of activity of TRH in hypophysectomized mice, suggests that TRH may also possess antidepressant value but by a mechanism independent of thyroid hormone action.

Note added in proof: Since this report was submitted for publication, Prange and Wilson have established in clinical studies with TRH that there is an immediate (within 2 hours) antidepressant effect in patients with unipolar depression (*10*).

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Arthropod Molting Hormone: Radioimmune Assay

Abstract. *A radioimmune assay for the arthropod molting hormone, ecdysterone, has been developed. The sensitivity of the assay is 200 picograms or 25 times the maximum sensitivity of the bioassay. Closely related steroids also bind the antibody, but with lower affinities.*

Ecdysterone (β-ecdysone, crustecdysone) induces molting in many arthropodan species (*1*). One technical problem that has hindered research related to ecdysterone is the lack of a precise physicochemical technique for quantifying tissue and circulating titers of this hormone. In the standard bioassay for crustecdysone either *Calliphora* (*2*) or *Musca* (*3*) is used as the assay organism, but like most bioassays it is relatively insensitive.

A number of laboratories have investigated a gas-liquid chromatographic method (GLC) for quantifying either α- or β-ecdysone (*4*). While these procedures are promising, the problems inherent in purifying biological samples for GLC as well as interfering side reactions in the derivatization of ecdysones tend to make this alternative less attractive at present.

Another technique for measuring steroid hormones, which are often present in vanishingly small quantities, is the radioimmune assay (*5*). By conjugating the hormone to a large carrier protein [such as bovine serum albumin (BSA)], the steroid is rendered haptenic and can elicit an immunogenic response. Since the specificity of the antibody response is dependent on the homogeneity of the haptene configuration, a derivative is first formed at one position, which is then reacted with the carrier protein. Derivatization of ecdysterone at the 6-keto function was chosen for a number of reasons. Theoretically the limit of detection by this method is dictated primarily by the specific activity of the labeled standard available.

Ecdysterone (40 mg) was converted to the oxime acetic acid ether in a 4 percent solution of aminooxyacetic acid (*6*) in pyridine, overnight, at 40°C. The identity of the oxime acetic acid ether derivative and its methyl ester was established by infrared and ultraviolet spectrum analyses and thin-layer chromatography in three different solvent systems (*7*). The oxime derivative was coupled to BSA by way of the isobutylchloroformate mixed anhydride intermediate. The protein-ecdysone conjugate was then dialyzed against running distilled water overnight and lyophilized (*8*).

The conjugate (4 mg) was suspended in equal volumes of Freund's complete adjuvant and saline and injected subcutaneously and intramuscularly into each of three New Zealand white rabbits. A 1-mg booster injection was administered in similar fashion 6 weeks after the initial injection. Blood was collected 9 days after the booster injection and analyzed for antibodies to crustecdysone. Control serum was collected from uninjected animals.

The antibody response was assayed according to a method modified from

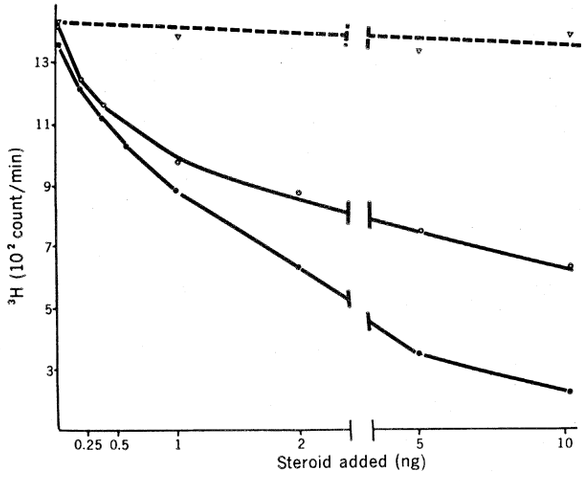

Fig. 1. Inhibition of [^{3}H]ecdysterone binding by rabbit antiserum in the presence of increasing amounts of various unlabeled steroids; (●), ecdysterone; (○), α-ecdysone; (△), 3β-hydroxy-5α-cholestan-6-one. All reaction tubes contained 1 percent antiserum and [^{3}H]ecdysterone (4000 count/min). Total volume was made up to 0.5 ml with borate buffer (*p*H 8.4). To facilitate precipitation of antibody-bound haptene, total serum concentration was brought up to a constant 10 percent in each reaction tube by the addition of control serum. Nonspecific binding of label by a 10 percent solution of control serum amounted to 150 count/min and has been subtracted from each of the above values.