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CONVENIENT PROCEDURE FOR EXTRACTION OF GONADOTROPIN-RELEASING HORMONE AND THYROTROPIN-RELEASING HORMONE FROM HYPOTHALAMIC TISSUE

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1. Introduction

Measurement of tissue concentrations of the hypothalamic hormones gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) is essential for studying their physiological roles, since reliable determination of the peripheral serum levels of these hormones has not yet been achieved [1,2]. Only minute amounts of GnRH and TRH are released into the hypothalamo-hypophyseal portal system, and these are greatly diluted in the general circulation. Moreover, these peptides have an extremely short half-life time [3,4]. Thus, at present, study of the fluctuations in the hypothalamic content of GnRH and TRH provides the only means of relating various physiological events to changes in the production and release of these hormones. The established procedures for quantitative extraction of hypothalamic hormones, however, are cumbersome as they involve acidification, readjustment of pH and solvent evaporation. This report presents an alternative, simple procedure for quantitative extraction of GnRH and TRH.

2. Materials and methods

2.1. *Dissection*

The medical basal hypothalamus (consisting mainly of the median eminence and the arcuate nucleus; approximate weight 4–6 mg) was dissected, according to de Groot [5], from 5-month-old, Wistar-derived male rats of the departmental colony.

2.2. *Extraction procedures*

Hydrochloric acid: Hypothalamic tissue from one rat was homogenized in 1 ml of 0.1 N HCl and centrifuged at 14 000 *g*. The supernatant was neutralized (1 N NaOH), recentrifuged and used for radioimmunoassay.

Methanol or acetic-methanol: The tissue was homogenized in 1 ml methanol or in a solution of 2 N acetic acid in methanol. After centrifugation at 14 000 *g*, the solvent was removed by evaporation and the residue dissolved in 0.01 M phosphate-buffered saline (PBS; pH 6.9) for the assay.

Boiled neutral extract: The tissue was immersed in 1 ml PBS, in Thomas tissue grinder vessels (size AA), which were placed in a boiling bath for 3 min; homogenization in the same medium was followed by reboiling for 3 min and centrifugation at 14 000 *g* for 10 min. The supernatant fraction was kept for the assay.

2.3. *Radioimmunoassay*

Amounts of GnRH and TRH extracted by the four procedures described, were determined by radioimmunoassay, using antisera developed in this laboratory [6,7]. Samples were assayed in duplicate and at several dilutions. LH and TSH were assayed using kits kindly provided by the NIAMD-NIH Rat Pituitary Hormone Program. Results are expressed in terms of the RP-1 reference preparations.

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2.4. *Bioassay*

Hemipituitaries of male rats were incubated for 2 h in 1 ml of Krebs-Ringer bicarbonate buffer containing 10 mg% glucose (KRBG). After change of medium the appropiate stimulants were added and incubations continued for 4 h. Amounts of LH and of TSH in the media were determined by radioimmunoassay.

3. Results

The concentrations of GnRH and TRH in rat hypothalamus, as determined by radioimmunoassay following the various extraction procedures, are summarized in table 1. Use of boiled neutral-PBS extracts yielded estimates of GnRH and TRH comparable to those resulting from the standard extraction procedures. The biological activity of GnRH and of TRH extracted by boiling, as compared to that of equivalent amounts of the corresponding synthetic peptides is shown in table 2. The extracted hormones are as potent in releasing LH or TSH as their synthetic counterparts.

Table 1
GnRH and TRH concentrations in the medial basal hypothalamus (MBH) of male rats, as estimated by use of four different extraction methods and an identical radioimmunoassay procedure

Extraction	GnRH ng/MBH	TRH ng/MBH
Boiled PBS	6.10 ± 0.26	4.86 ± 0.15
Methanol	5.81 ± 0.34	4.68 ± 0.23
Acetic-methanol	5.90 ± 0.36	4.45 ± 0.30
Hydrochloric acid	5.88 ± 0.37	4.48 ± 0.11

Mean ± S.E.M. for 12 tissue samples per group.

4. Discussion

Data have been presented to show that GnRH and TRH can be quantitatively extracted by boiling tissue homogenates in an aqueous neutral solution; both hormones fully retain their biological and immunological activites. This short and simple technique makes it possible to use the extraction medium directly for radioimmunoassay or bioassay, eliminating the need for further manipulations such as neutralization or evaporation. Omitting the boiling step leads to poor yields, e.g.: less than 10% for GnRH, as observed by Shin and Howitt [8]. This finding may be explained by enzymic degradation of the peptides [9,10] and/or by their inaccessibility: the hormones may be bound to a protein carrier [11] or confined in vesicles [12]. Boiling inactivates degrading enzymes and may disrupt membrane structures or non-covalent protein-peptide binding. The biological and immunological properties

Table 2
Biological activity of hypothalamic GnRH and TRH extracted by boiling

Preparation	Hormone released to the medium (μg/ml)	
	LH	TSH
Control	0.59 ± 0.08[a]	13.0 ± 1.7
Synthetic GnRH, 1 ng	5.10 ± 0.46	–
Hypothalamic GnRH, 1 ng[b]	5.53 ± 0.32	–
Synthetic TRH, 1 ng	–	138.0 ± 9.3
Hypothalamic TRH, 1 ng[b]	–	119.0 ± 9.8

[a] Mean and standard error of the mean for 6–12 determinations.
[b] As quantitated by radioimmunoassay.
Hypothalamic peptides were incubated for 4 h with hemipituitaries in 1 ml of KRBG. LH and TSH were determined by radioimmunoassay.

of the hypothalamic hormones, however, are unaffected by boiling.

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