BIOASSAY OF THYROTROPIN USING ISOLATED PORCINE THYROID CELLS

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1. Introduction

Numerous methods of bioassaying thyroid stimulating hormone (TSH) have been described and for most of them abandoned. The most widely used at the present time are: (1) the in vivo bioassay first proposed by Adams and Purves [1] in the guinea-pig and later applied to the mouse by McKenzie [2], which measures in thyroid hormone-pretreated animals the secretion of protein-bound ^{131}I in the blood; (2) an in vitro method estimating the release of ^{131}I from incubated thyroid slices. The latter procedure was initially proposed by Bakke and Lawrence [3] and later modified and improved by Kirkham [4] and Desbarats-Schönbaum et al. [5,6]. Both methods are sensitive being able to detect 0.1 to 10 nM concentrations of TSH but their precision is limited (index of precision about 0.2). In addition, responses to TSH from different species are not parallel precluding comparison of their specific bioactivity. Methods of TSH assay have been recently reviewed [7,8].

In this letter, we describe the use of isolated porcine adult thyroid cells to assay the biological potency of TSH. The method is based on the capacity of the hormone to stimulate the reorganization of the cells into follicles in culture conditions [9,10] and the parallel ability of the reorganized cells to concentrate iodide and iodinate thyroglobulin [11,12].

2. Materials and methods

2.1. *Standard method of TSH bioactivity measurement*

Isolated thyroid cells were obtained from porcine glands of adult animals by a discontinuous trypsinization procedure [9]. Freshly isolated cells were suspended in Eagle's medium pH 7.0 containing 20% (v:v) calf serum, streptomycin sulfate (50 μg/ml) and penicillin (200 U/ml) at a concentration of 3×10^6 viable cells/ml, and seeded in 35 mm plastic Petri dishes (1 ml/dish). 50 μl of TSH dissolved in the same medium were added to give a final concentration of 100 pg to 10 ng/ml. Controls were supplemented with hormone-free medium. After 4 days incubation at 35°C in humidified 95% air–5% CO_2, 50 μl of $Na^{127}I$ traced with 2 μCi carrier-free $Na^{125}I$ in medium (50 μM final concentration) were added to each dish. Incubation in the same conditions was continued for 6 hr. The dishes were then placed on crushed ice, in incubation medium was carefully removed with a Pasteur pipette and the cell layer was washed 3 times with 1 ml 0.14 M NaCl. The cells were detached from the support by the addition of 1 ml 0.1 N NaOH. After 15 min of gentle rotatory shaking at room temperature, the cells were transferred into a counting tube. The procedure was repeated once and the radioactivity of the pooled suspensions was estimated in a well-type scintillation

spectrometer. This procedure allowed a complete recovery of the radioactivity contained in cells. All steps were performed in sterile conditions. Assays of each TSH concentration and controls were performed in triplicate or quadruplicate.

Estimation of radioactivity associated with protein in the cell layer was carried out by precipitation with TCA. One ml of 30% (w:v) TCA solution was added to the suspension (2 ml) of NaOH-detached cells. After homogenization and centrifugation, the pellet was washed 3 times with 10% TCA solution and counted.

2.2. *TSH preparations*

Porcine and bovine TSH preparations were described elsewhere [13]. Their specific biological activity in the McKenzie bioassay amounted to 34 and 22.5 IU/mg, respectively. Human TSH was prepared according to chromatographical procedures referred to in [13]. Its biological specific activity could not be determined in vivo as the bovine International reference preparation for TSH and human TSH give non-parallel slopes for their dose-response curves in the McKenzie's assay [2]. Our human TSH preparation was found 3.16 times more potent in radioimmunoassay than human TSH Research Standard A.

2.3. *Calculations*

The results are expressed as mean ± SEM or as mean of closely agreeing replicates. The index of precision is defined as $\lambda = s/b$ with s, estimated standard deviation per response, and b, slope of the log dose-response regression. A mol. wt of 28 000 for porcine TSH was used for calculations.

3. Results

Preliminary experiments showed that in the presence of porcine TSH (40 mU/ml), porcine thyroid cells incubated at 35°C in plastic Petri dishes reorganize into follicles. Optimal adhesion to the support and maximal follicular reorganization were observed 4 days after the onset of incubation. At that time, light microscopic examination of cells incubated with increasing porcine TSH concentrations (from 0.25 to 10 ng/ml) showed a parallel increase in histiotypic reassociation and incorporation of ^{125}I into the cell layer (table 1).

Table 1
Effect of increasing concentrations of porcine TSH on the reorganization of thyroid cells into follicles and on the incorporation of $Na^{125}I$ in the cell layer

pTSH	Cells in histiotypic organization[a]	Cells in monolayer	Radioactivity in the cell layer
ng/ml		% total surface of support	cpm
0	–	100	5100
0.25	–	90	8500
0.50	+	90	15 800
1.00	+	70	34 900
1.25	+	50	42 600
2.50	++	40	50 000
5.0	++	30	66 000
10.0	+++	5	70 000

[a] –, no follicles; +, ++ and +++: less than 5, 5 to 20 and more than 20 follicles per field.
$Na^{125}I$ added: 400 000 cpm/dish

The time-course of ^{125}I incorporation into the cells is described in fig.1 for three porcine TSH concentrations. A 6-hr incubation time was chosen for standard assays. At that time the increment of ^{125}I incorporation with function of time is enough reduced so that a small error made on time measurement will not affect appreciably the total amount of ^{125}I incorporated.

In standard conditions, the uptake of ^{125}I by cells incubated for 4 days is linearly related to the logarithm of porcine TSH concentrations comprised between 0.25 and 5 ng/ml or 9 and 180 pM (fig.2).

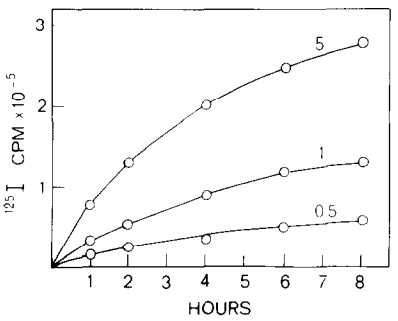

Fig.1. Time-course of ^{125}I incorporation into the cell layer. Cells were incubated from the onset of culturing in the presence of 0.5, 1 and 5 ng porcine TSH/ml, respectively. Each value is the mean of closely agreeing duplicates.

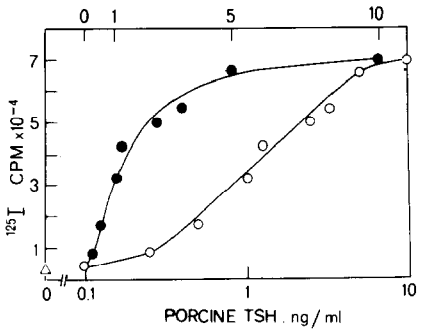

Fig.2. Relation between porcine TSH concentration and Na ^{125}I uptake by isolated porcine thyroid cells after 4 days incubation at 35°C in culture conditions. (•) algebraic scale (upper abscissa); (○) logarithmic scale (lower abscissa); (△) control without TSH. Experimental conditions in Methods.

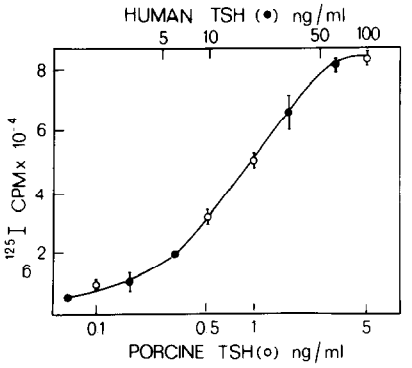

Fig.3. Comparison of the dose–response curves obtained with porcine (○) and human (•) TSH. Each value is the mean of assays in quadruplicate. Bars, SEM.

The characteristics of the response are the following: (1) it is a saturable process with respect to TSH concentration; (2) its amplitude is large since 100% response corresponds to an amount of incorporated radioactivity about 30 times higher than that of controls without hormone; (3) sensitivity is good, a TSH concentration of 18 pM giving 20% of maximum response; (4) the index of precision (λ) is 0.06; variance analysis showed that the linear regression of responses on log-dose is highly significant ($F = 659$ for $F_{0.01} = 12.25$) non-linear terms being not significant ($F = 2.36$ for $F_{0.05} = 3.97$).

The response may vary from batch to batch of isolated cells but the results expressed as percent of maximum response vs log-TSH dose always gave parallel lines. In 10 experiments, half-maximum stimulation of ^{125}I uptake was comprised between 0.9 and 1.4 ng/ml [43 ± 10 pM (mean ± SEM)].

Table 2
Protein-bound ^{125}I in porcine thyroid cells stimulated by increasing concentrations of porcine TSH

pTSH	Total radioactivity in the cell layer	TCA-insoluble radioactivity	
ng/ml		cpm	% total
0.1	9300	7300	78
0.5	34 200	27 000	79
1.0	50 200	38 400	76
5.0	82 900	65 200	79
50.0	81 300	64 100	79

Each figure is the mean of closely agreeing triplicates

About 80% of the total radioactivity incorporated into the cell layer are in the form of iodoprotein (thyroglobulin) as shown by precipitation with TCA (table 2).

Highly purified TSH from different mammalian species were compared. Fig.3 shows the dose-response relation observed with the human and porcine hormones. The curves are superimposable but on a weight basis human TSH is about 20 times less active than is porcine TSH. Same experiments performed with bovine TSH equally disclosed parallelism of the dose-response curves and the same value of maximum stimulation. In the system, bovine TSH was about 5 times less active than porcine TSH. Statistical analysis of the responses to the three hormones for parallelism is shown in table 3.

This method was applied to the estimation of the biological activity of ^{125}I-labeled porcine TSH of high specific radioactivity used for receptor binding studies [14]. The hormone was labeled using the lactoperoxidase method of [^{125}I] iodide oxidation [15]. Preparations of [^{125}I] TSH containing 1.5 to 2 iodine atoms per hormone molecule (2.0 to 2.6 Ci/μmol) were compared to the same TSH solution from which the aliquot to be iodinated was taken. A typical dose–response curve is shown in fig.4. However, sometimes and for still unknown reasons, the

Table 3
Comparison of TSH from different species

Origin of hormone	F_{LR}	F_{NLT}	Slope of the regression line	Inverval of confidence $p = 0.95$
			cpm/log-dose	cpm/log-dose
Hog	70[a]	< 1[b]	8800	± 2400
Man	116[a]	2[b]	9730	± 2800
Ox	175[a]	< 1[b]	10 730	± 2300

[a] highly significant ($> F_{0.01}$)

[b] non significant ($< F_{0.05}$)

F_{LR}, variance due to the linear regression/variance of error

F_{NLT}, variance due to non linear terms/variance of error

response to radio-iodinated hormone was not paralled to that given by the native hormone but the same level of maximum response was always obtained for the same concentration of both modified and intact hormones.

4. Discussion

The uptake and organification of radioactive iodide by isolated porcine thyroid cells incubated for 4 days in the presence of serial dilutions of TSH provides a sensitive, accurate and reproducible method of assaying TSH bioactivity.

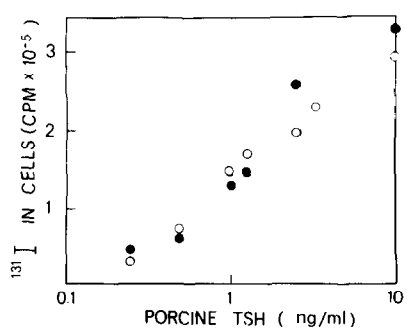

Fig.4. Comparison of the dose–response curves of porcine TSH (○) and its ^{125}I-labeled derivative (●). The ^{125}I-labeled TSH preparation had a specific radioactivity of 2.2 Ci/μmol corresponding to 1.7 iodine atoms incorporated per mol of hormone. In this type of experiments $Na^{125}I$ added to cells was replaced by $Na^{131}I$.

As little as a 9 pM porcine TSH concentration can be detected (or 0.01 mU/ml on the basis of a specific activity of 40 U/mg as measured by the McKenzie bioassay). The in vitro assay using the release of radioactive iodine by guinea-pig thyroid slices can usually detect 0.017 mU/ml and the in vivo assay in the mouse 0.25 mU/ml.

In contrast to the limited precision of the avialable methods (index of precision about 0.2; see [7] for a review) the described assay using isolated thyroid cells has comparatively an excellent precision ($\lambda = 0.06$ to 0.08). In addition, reproducibility from batch to batch of cell is good and easier to obtain since no pretreatment of the animals is needed.

Another advantage of the method described in this letter concerns the possibility to *compare* the specific activity of TSH obtained from different species. Indeed using highly purified human, bovine and porcine TSH, the dose–response curves showed parallelism and identical levels of maximum response. With the test using porcine cells, bovine and human TSH had specific activities 5 and 20 times lower than porcine TSH possibly indicating a high degree of species specificity of the receptor sites for TSH. However no definitive conclusion on this point can be drawn before comparison with human and bovine cells. These experiments are in progress.

Acknowledgements

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