The presence of LHRH-like receptors in Dunning R3327H prostate tumors

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Quantitative analyses of LH-RH-like membrane receptors were performed in five tumors from the transplantable Dunning R3372H rat prostatic adenocarcinoma. The binding of D-Trp^6-LH-RH, an agonist of LH-RH, was observed in all 5 tumors. The antagonist [Ac-Dp-Cl-Phe^{1,2},D-Trp^3,D-Lys^6,D-Ala^{10}]-LH-RH was bound to 4 tumors. The apparent equilibrium dissociation constant (K_d) for D-Trp^6-LH-RH receptor was from 2.6–3.9 $\times$ 10^{-10} M. The apparent equilibrium B_{max} values (maximum number of binding sites) were from 17.2–86.0 fmol/mg membrane protein for D-Trp^6-LH-RH receptor. The K_d for the antagonist was from 2.4–2.7 $\times$ 10^{-10} M and the B_{max} values were from 35.5–66.0 fmol/mg membrane protein. Similar binding studies performed in 6 normal rat prostates showed no binding capacities.

Dunning R3327H prostate tumor *Membrane protein* *LH-RH analog binding*

1. INTRODUCTION

We have observed an inhibition of the growth of Dunning R3327H prostate tumor after treatment with agonistic [1] as well as antagonistic [2] analogs of LH-RH. Although prostate tissue is not normally a target tissue for LH-RH action, we have found LH-RH-like receptors on plasma membranes from Dunning R3327H prostate tumor, which bind agonistic as well as antagonistic analogs of LH-RH. The present report describes our findings.

2. MATERIALS AND METHODS

[Ac-Dp-Cl-Phe^{1,2},D-Trp^3,D-Lys^6-D-Ala^{10}]-LH-RH, an antagonistic analog of LH-RH, was synthesized in our laboratory by solid-phase methods [3]. D-Trp^6-LH-RH was also synthesized by solid-phase methods and supplied by Debiopharm SA (Lausanne).

2.1. *Tissue preparation*

Normal (Male Copenhagen $\times$ Fisher)F_1 rats and rats bearing the androgen-dependent, well-differentiated R3327H Dunning rat adenocarcinoma were kindly provided by Norman Altman (Papanicolaou Cancer Research Institute, Miami FL). Tumors were palpable 160 days after transplantation, and rats bearing tumors $\geqslant 25$ mm^3 were selected for the study. Rats were decapitated and the ventral and dorsal prostates or prostate tumors rapidly removed, cleaned of necrotic or connective tissue, washed with 0.25 M sucrose and placed on ice. In some cases normal pituitary tissue was removed to compare the binding ability of the pituitary membranes and the prostate tumor membranes. Tissues were frozen and kept at -70°C until assayed for LH-RH receptors.

2.2. *Membrane preparation*

The tissue was homogenized with Dounce homogenizer at 4°C in assay buffer consisting of 10 mM Tris–HCl (pH 7.4), 1 mM DTT, 0.1% BSA and centrifuged for 10 min at 1000 $\times$ g. The supernatant was then centrifuged for 20 min at 20000 $\times$ g. The pellet was suspended in assay buf-

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fer, centrifuged again as above and resuspended in buffer. The presence of plasma membranes in this fraction was assessed by the distribution of enzymic activities: adenylate cyclase [4], 5′-nucleotidase [5] and phosphodiesterase [6].

2.3. *Iodination of LH-RH analogs*

[D-Trp^6]-LH-RH and [Ac-Dp-Cl-Phe^{1,2},D-Trp^3,D-Lys^6,D-Ala^{10}]-LH-RH were iodinated by a solid phase method with iodogen [7]. The reaction mixture was applied on a Sephadex SP-C25 column (2 × 1 cm) equilibrated to pH 3.0 with 0.01 mol/glycine–HCl buffer. The same buffer was then used to elute unreacted iodide. The peptide peak was eluted with 0.5 mol/l phosphate buffer (pH 7.5). The labeled peptide fraction was rechromatographed if necessary on Sephadex G-25. The specific activity of the iodinated analogs, measured by self-displacement in the radioreceptor assay, was 1000–1290 μCi/μg for the agonist and 850–1050 μCi/μg for the antagonist. The maximum binding of labeled analogs was estimated by using an excess of rat pituitary membranes and comparing the maximum binding of these radioligands to that observed in membranes from Dunning R3327H prostate tumor. The maximum binding of labeled analogs was 60–65% of counts added for both analogs. Receptor affinity and binding capacity were expressed as fmol analog bound/mg membrane protein.

2.4. *Binding assays*

The assays were conducted in triplicate in BSA-precoated polypropylene tubes. The incubation mixture contained assay buffer (10 mM Tris–HCl (pH 7.4), 1 mM DTT and 0.5% BSA), the ^{125}I-labeled agonist or antagonist of LH-RH ~50000 cpm, varying amounts of unlabeled analogs, and 75–200 μg membrane protein in 0.3 ml final vol. The tubes were incubated for 90 min at 4°C. The reaction was stopped by adding 1 ml ice-cold assay buffer, and the receptor-bound ligand was separated from the unbound by filtration under vacuum through Whatman GF/C filter. Of the added tracer, ~8% was bound in the absence of unlabeled hormone. The non-displaceable binding, estimated in the presence of 80 ng unlabeled analog, was < 2% of the total counts added.

2.5. *Protein measurement*

Protein was determined following [8] as modified [9]. Protein samples were analyzed at 3 dilutions in duplicate.

3. RESULTS

3.1. *Optimal concentration of membrane proteins*

Optimal concentration of membrane proteins by radioreceptor assay is shown in fig.1. The points represent the mean of duplicates. A linear relationship was observed between the amount of particulate protein (up to 150 μg) and the specifically-bound radioactivity (with 0.9 pmol tested LH-RH analogs).

3.2. *Characterization of LH-RH-like receptor in Dunning R3327H rat prostate tumor*

The specific binding of LH-RH analogs to membrane proteins was maximal at 4°C, and steady state binding was reached at 90 min and remained stable for an additional 80 min. When the temperature was increased to 20, 25 or 37°C, the association rate was increased but the maximum binding was reduced.

After establishing optimal conditions for the radioreceptor assay, the binding inhibition curves [10] were performed. An initial low concentration of ^{125}I-D-Trp^6-LH-RH (15 fmol/tube) and increasing amounts of both labeled and unlabeled agonist gave similar half-maximal displacements (150 fmol/tube for labeled analog and 100 fmol/tube for unlabeled analog). The data from inhibition curves were analyzed by Scatchard plot and gave $K_d = 3 \times 10^{-10}$ M for labeled analog,

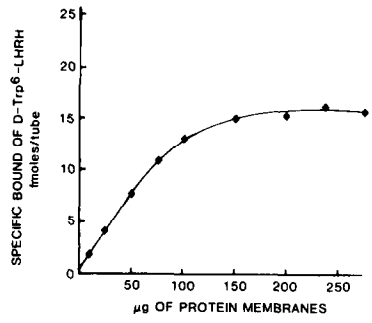

Fig.1. Specific binding of ^{125}I-D-Trp^6-LH-RH to increased concentration of membrane proteins from Dunning R3327H prostate tumors.

and 2.5×10^{-10} M for unlabeled analog. Similar results have been obtained with the antagonist.

The characterization of the LH-RH-like receptor in Dunning R3327H prostate tumor was performed in two kinds of experiments with unsaturated amounts of membrane protein:

(1) Varying amount of cold analog/tube (12.5–640 pg for agonist and 13.5–685 pg for antagonist, with constant amount of ^{125}I analog/10 pg);

(2) Varying amounts of ^{125}I-labeled analog (23–312 pg for agonist and 23–347 pg for antagonist).

In all these experiments 75 μg and 150 μg membrane protein/tube were used. Scatchard plot analyses of these two kinds of experiments gave similar values for equilibrium dissociation constant and B_{max} (fig.2). Subsequent determinations of binding capacities for agonist and antagonist were performed by using saturation analysis.

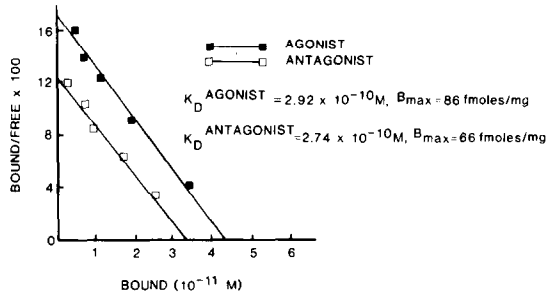

Fig.2. Scatchard plots of ^{125}I-D-Trp^6-LH-RH (■—■) and ^{125}I-[Ac-Dp-Cl-Phe^{1,2},D-Trp^3,D-Lys^6,D-Ala^{10}]-LH-RH (□—□) with Dunning R3327H prostate tumor membrane proteins.

Fig.3 represents the saturation curve of specific binding (difference between the total and nonspecific binding) for each point of the curve. The inset Scatchard plot from saturation curves showed that the dissociation constants and the

Table 1

Binding of LH-RH analogs to membranes of normal rat prostate and Dunning R3327H prostate tumors

		[D-Trp^6]-LH-RH			[Ac-D-p-Cl-Phe^{1,2},D-Trp^3,D-Lys^6,D-Ala^{10}]-LH-RH		
	No. rats	$K_d \pm$ SD (10^{-10} M)	Capacity in fmol/mg membrane protein		$K_d \pm$ SD (10^{-10} M)	Capacity in fmol/mg membrane protein	
			Scatchard plot	Saturation analysis		Scatchard plot	Saturation analysis
Normal							
Ventral	6		No binding			No binding	
Dorso-lateral	6		No binding			No binding	
Tumor							
Tumor 1	1	2.6 ± 0.3 n = 9	49.0	38.4	2.4 ± 0.28 n = 8	35.5	30.5
Tumor 2	1	3.9 ± 0.3 n = 7	64.0	56.1	3.2 ± 0.3 n = 7	53.1	45.7
Tumor 3	1	2.98 ± 0.2 n = 5	86.0	82.5	2.74 ± 0.2 n = 5	66.0	66.5
Tumor 4	1	2.8 ± 0.3 n = 5	17.2	13.2		No binding	
Tumor 5	1	3.0 ± 0.3 n = 10	74.0	64.8	2.7 ± 0.25 n = 8	60.5	53.9

n = Number of points in the Scatchard plot used

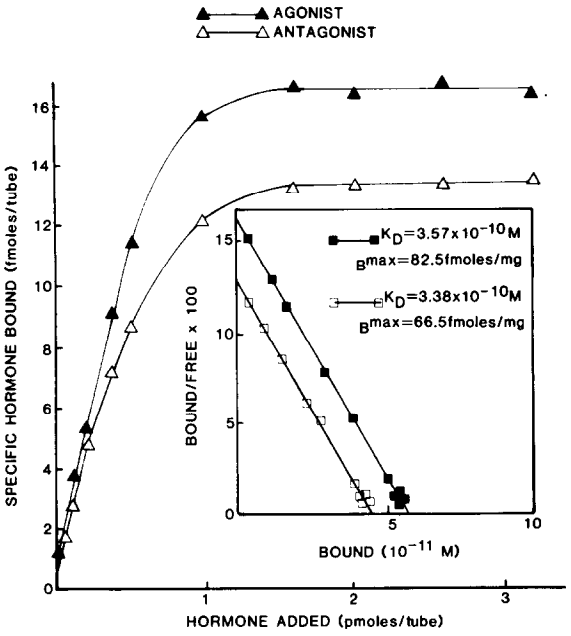

Fig.3. Saturation curves of LH-RH agonist (▲—▲) and antagonist (△—△) binding to prostate tumor membrane proteins. Inset: Scatchard plot from both saturation curves, (■—■) agonist and (□—□) antagonist.

Table 2

Lack of effect of TRH and somatostatin on binding of LH-RH analogs (fmol/mg membrane protein) from Dunning R3327H prostate tumor

[Peptide] (M)	Agonist (Means ± SE)	*p*	Antagonist (Means ± SE)	*p*
TRH	82.5 ± 0.5		66.5 ± 0.45	
10^{-8}	82.1 ± 0.44	NS	65.8 ± 0.5	NS
10^{-6}	81.5 ± 0.48	NS	65.7 ± 0.35	NS
Somato-statin				
10^{-8}	81.8 ± 0.65	NS	65.8 ± 0.48	NS
10^{-6}	82.4 ± 0.75	NS	66.7 ± 0.56	NS

The binding was determined as in section 2. The data presented here are means ± SE of triplicate incubations with 960 fmol/tube for agonist or antagonist of LH-RH with 200 μg membrane protein. Differences between means were analyzed by Student's *t*-test or one-way analysis of variance with Duncan's multiple range test

binding capacity calculated from saturation analysis were in good agreement with binding capacities calculated from Scatchard plots (table 1). The binding of LH-RH analogs to membrane proteins from prostate tumors was not affected by TRH and somatostatin (table 2).

4. DISCUSSION

Our previous observations that administration of agonistic and antagonistic analogs of LH-RH induced regression of rat prostate tumors were probably linked to the inhibitory effect of LH-RH on the levels of sex steroids [1,2]. Others have demonstrated a direct action of agonistic LH-RH analogs on testes and ovaries, and the presence of LH-RH receptors in these tissues [11–17]. The present work indicates that Dunning R3327H tumor tissue possesses binding capabilities for both LH-RH agonist and antagonist. Normal prostate tissue did not bind these LH-RH analogs. The binding of LH-RH antagonist to prostate tumor tissue demonstrates an extrapituitary effect of antagonistic analogs of LH-RH. To characterize the LH-RH-like receptor we used Scatchard plot analysis with unsaturated amounts of membrane proteins and a saturation curve analysis. The maximum binding capacities in both sets of experiments were similar for agonist (86 fmol/mg protein *vs* 82.5 fmol/mg protein), and for antagonist (66 fmol/mg protein *vs* 66.5 fmol/mg protein). The LH-RH-like receptor in Dunning R3327H rat prostate tumor is a single class receptor with K_d 2.6–3.9 × 10^{-10} M for agonist and 2.4–2.7 × 10^{-10} M for the antagonist. An apparent absence of high affinity binding sites in prostatic tissue of normal rats in our assay suggests that carcinogenic transformation induces changes in membrane structure and composition, resulting in the appearance of LH-RH-like binding sites. Which components of the ectopic LH-RH-like receptor are responsible for the binding of LH-RH analogs in Dunning R3327H prostate tumor, and whether prostate tumor receptors are similar in structure and composition to those of the pituitary, is under investigation.

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