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RADIO-RECEPTOR ASSAY (RRA)

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Cellular signaling constitutes an important array of information flow in biological systems. It has been revolutionary conserved from microorganisms to humans. All such organisms use one or the other form of signal(s) to generate the desired response. These signals include a wide variety of molecules starting from amino acids and their derivatives to proteins/peptides on one hand and the steroid and other lipid derivatives on the other. The hydrophilic signals (amino acid derivatives and proteins) being water soluble, can not cross the plasma membrane and hence act by binding to specific membrane bound receptors. These receptors are mostly coupled to transducer G-proteins which influence the amplifier enzymes to reduce a variety of second messengers (camp, cGMP, IP3, DAG, No, Ca_2^+, etc). These messengers modify the effectors proteins and enzymes to elicit the cellular response. On the other hand, the lipophilic signals (steroid and their derivatives) , being lipid soluble, can cross the plasma membrane and bind to specific intracellular receptors, located either cytosol or in the nucleus of a target cell. Steroid recepto (S-R) com-

plexes undergo activation (transformation) and interact to specific DNA sequences, ... as steroid responsive elements (SREs). This interaction modulates the cognate gene expression by enhancing the synthesis of mRNAa and related proteins, thus producing an appropriate cellular response. Besides these genomic actions of steroid hormones, recently, non-genomic actions of steroid hormones are also appreciated. They have been found to modulate cell-surface receptors in selected examples. These membrane bound receptors may either be specific to steroids or the steroids may bind to other protein/peptide hormone receptors. Interaction to the later may modulate the signaling of the respective protein/peptide hormone. Since the physiological responsiveness of cells/tissues to hormones is mostly dependent on the level/properties of their cognate receptors, the measure of receptor concentration and property is of utmost importance in deducing the functional correlates of cellular signaling. It is therefore, envisaged to know how to assay the receptors using their cognate radioactive hormone, termed a **radio-receptor assay (RRA)**

Physical parameters for hormone-receptor interaction

Hormone-binding reaction, in which, one molecule of receptor (R) bindr to one molecule of hormone (H) , can be written as :

$$H + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} HR$$

k_1 = rate of association
k_2 = rate of dissociation

Under equilibrium condition

$$\frac{[R][H]}{[HR]} = \frac{k_1}{k_2} = k_d \quad (K_d \text{isdissociationconstant})$$

Since the free receptor (R) equals the total receptor (Rt) minus that which has been bound to hormone (HR), [R=Rt - HR], the equilibrium equation can be written as follows:

$$K_d = \frac{[H][R]}{HR} = \frac{[H][R_t - HR]}{HR} \qquad k_d[HR]=[H][Rt-HR]$$

$$\frac{[HR]}{[H]} = \frac{[R_t - HR]}{k_d}$$

$$\frac{[HR]}{[H]} = \frac{R_t}{k_d} - \frac{HR}{k_d}$$

$$\frac{[HR]}{[H]} = (-1/k_d)[HR] + \frac{[R_t]}{k_d}$$

Since [HR] = bound hormone- receptoe complex & [H] = free hormone

$$B/F = (-1/k_d)\, B + [R_t]/k_d$$

This equation can be plotted on a straight line where the slope = $-1/k_d$ or $-Ka$; X-intercept= R_t; Y-intercept = R_t/k_d

Experimental protocol

1. Varying conc. of labeled [H]

1 2 3 4 5 6 7 8

(2-120 nM) **Hot**

2. Varying conc. of labeled [H] +500-1000x more cold [H]

1 2 3 4 5 6 7 8

(2-120 nM) **Hot** +(1-60 µM) cold

3. Fixed amount (100 ml) of Receptor preparation

(100 µl)

Incubate for approx. time (30 min. – 4 hr)

4. Add 50 µl of Dextran-coated Charcoal (can also be done by "Fibre Filter Assay")

Wait for 5-10 min

5. Spin at 2000 rpm # 5 min

Take 100 µl of aliquot from each tube (1-8) and add 4-6 ml of counting cocktail and mix well before counting in LSC.

Conc. of Hormone taken (nM)	Total [H]	HC	Conc.[H]	Conc.[HC]	Specific bound [B] (H-HC)	A-B (F)	Free	B/F	Plot B/F=Y B=X
2									
5									
10									
20									
40									
80									
120									

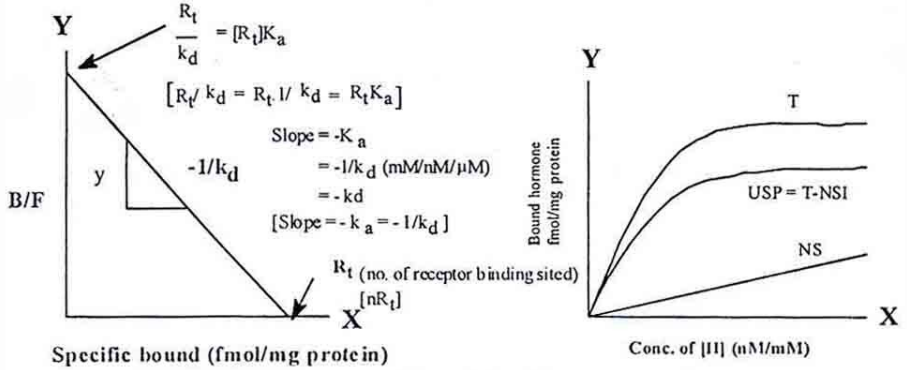

Fig: Scatchard plot for determining the concentration and affinity of recepteo for hormone

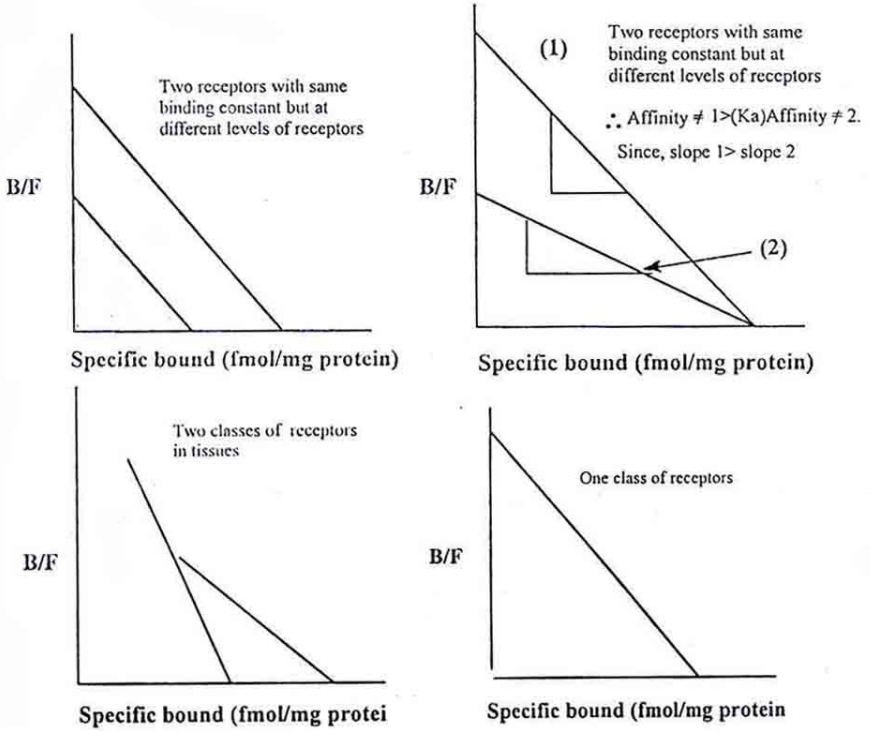

Fig: Several uses of Scatchard plot in examining tissues with more than one class of receptors for a given hormone in different tissues