RADIOIMMUNOASSAY CALIBRATION OF TRACER USING RADIOISOTOPE LABELS

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ABSTRACT

Twenty one days old, juvenile, male and females rats were administered GHRP-6 and its potent analogues, 96/185 and 96/257. Compounds were given at two doses of 0.3 and 1.0 mg/kg body subcutaneously. The control group received an equal volume of saline. The blood was collected from the choriod plexus at time intervals of 0, 5, 15, 30 and 45 minutes after the administration of the peptides, serum was separated by centrifugation stored at -700C until assayed. Serum GH concentrations at different periods were measured by a double antibody radioimmunoassay methods sing materials supplied by NIDDK (National Hormone Distributions Program) NIH, Bethesda, USA. Al values were compresses in terms of NIDDK reference preparation rat GHRP-1 as mg/ml of serum

Key words: juvenile, administered, volume, centrifugation, antibody

INTRODUCTION

In all types of immunoassays, it is very necessary to have a mean for determining the distribution between the bound and free fractions. Hence for this purpose, one of the components of the system must be labelled this 'tracer' may be the labelled antigen in a traditional radioimmunoassay system. Any substance which can be measured accurately by simple methods at levels less than that of physicochemical or other bio-assay methods for the measurement of theligand can act as a label. A radioactive isotope is the most widely used label. The nucleus of an atom consist of two main particles proton and neutron. The number of protons is the atomic number of the atom and determines the element's chemical characteristics. In some elements there is a difference in the number of neutrons but the number of protons remains the same. These are called 'isotopes'. Some isotopes have an unstable nucleus and these are known as 'radioactive isotopes'. Their nucleus transform to a more stable state by emitting energy in the form of particulate or non-particulate electromagnetic vibration.

a). Rat Growth Hormone Antigen.

Highly purified for iodination purpose, to be used as radio labbeled antigen.

b). Rat Growth Hormone Antiserum (monkey)

Reconstituted in 0.4ml, in 2% normal monkey serum in phosphosaline buffer ((PSB). Thisprovides a dilution of 1:50. Aliquotes of 25 µl were prepared and stored frozen at -70^0C until used.

c). Rat Growth Hormone Reference Preparation

Used as "Standard" for displacement curve, one ampoule consisting of 10 µl is reconstituted in 1 ml distilled water. Aliquotes of 25 µl of this 10 µl per ml solution were then prepared and stored frozen at-70^0C until used.

d). Anti-monkey gamma globulin

Dissolved in 5 ml of TDW. Aliquotes of 0.5 ml were prepared and stored frozen at -70^0C until used.

All the above reagents were used as per manufacturer's instructions. However, to monitor optimal antibody dilution, for use in subsequent assay antibody dilutions were titrated using "ELISA". The protocol was accordingly designed.

REVIEW OF LITERATURE

The rate growth hormone antigen supplied by Dr. A. F. Parlow (Harbor-UCLA Medical Centre, Torrance, USA) was iodinated following the Lodogen method used by Fraker & Speck (1978) with slight modifications. Sephadex G-25 swelled in TDW overnight and packed into a syringe column.

It was then washed with excess of PBS followed by wash with about 25-30ml of PBS containing BSA (0.1%). The column packed syringe was fixed in a clamp.

MATERIAL AND METHOD

Preparation of Iodogen Tubes

25µl aliquots of a chloroform solution containing Iodogen (0.1 mg/ml) was taken in an eppendorf-tube. The eppendorf tube was rotated in a manner to that a thin film of this solution was formed on the walls of the eppendorf tube.

25µl of 1 mg/ml solution of rat growth hormone antigen and 1 mci of I^{125} were added to the Iodogen tube. The reaction was allowed at room temperature for ten minutes with intermittent shaking. The remaining solution in the column was allowed to flow down. The reaction was stopped by adding 0.5 ml of PBS. The entire reaction mixture from the Iodogen tube was then loaded onto the bed of the column. Ten eluates of 50025µl aliquots eluted with PBS (BSA) were collected in eppendorf tube. Then 10 µl content from each of these eppendorf tubes '1' to '10' was taken in 10 different counting tubes meant for gamma counting. Counts per minutes for each tube were determined. Elutes containing maximum counts were selected and rest were discarded.

In another set of each eppendorf tube 0.5 ml of 0.1% BSA-PBS, 10 µl of the fraction from the respective eppendorf tubes and 0.5 µl of TCA was taken. This mixture was allowed to stand for 10 minutes at room temperature. They were then centrifuged at 10,000 rmp for 5 minutes. Both the supernatant and sediment were collected in different counting tubes and counts per minute were taken for all tubes/ percentage labelling was then calculated. Fractions showing about 85-92% labeling were selected and stored at 4^0C for further use in RIA.

Equipment's used of RIA.

1. A set of autoclavable micro-pipettes capable of accurately and precisely dispensing volumes from 5 µl, 10-100 µl and 100-1000 µl.
2. Glass assay tubes of 75 x 12 mm size.
3. Plastic racks capable of holding approximately 100 assay tubes as well as standard solutions and antibody dilutions were used.
4. A standard laboratory vortex mixer.
5. Refrigerated centrifuge.
6. Gamma counter: LKB wallac 1282 compugamma counter.

ii). Time Course Experiments

Twenty one days old, juvenile, male and females rats were administered GHRP-6 and its potent analogues, 96/185 and 96/257. Compounds were given at two doses of 0.3 and 1.0 mg/kg body subcutaneously. The control group received an equal volume of saline. The blood was collected from the choriod plexus at time intervals of 0, 5, 15, 30 and 45 minutes after the administration of the peptides, serum was separated by centrifugation stored at -70^0C until assayed. Serum GH concentrations at different periods were measured by a double antibody radioimmunoassay methods sing materials supplied by NIDDK (National Hormone Distributions Program) NIH, Bethesda, USA. Al values were compresses in terms of NIDDK referencepreparation rat GHRP-1 as mg/ml of serum. The effects of GHRP-6 and GHRP antagonists on the growth hormone release stimulated by GHRP-6, its potent analogue 96/257 and GHRP were studied. Experiments were performed on twenty-one days of rats of either sex. The animal were procured from the CDRI animal house and kept at standard laboratory conditions. The food and water was served ad libitum. The animal were divided into six groups with 3 animals in each group. Separate group of animals were treated with 1 mg/kg dose of GHRP-6 or peptide 96/257 or GHRH for their effect on GH release. Other groups of animals received same dose of GHRP-6, peptide 96/257 or GHRH and then challenged with GHRP-6 or GHRH antagonists. Fifteen minutes after the administration of the peptides, blood was collected from the choroid plexus and serum separated. These samples were stored at 70^0C until assayed. A control group with saline treated rats was also run concurrently. Attempts were also made to analyze the possible

second messenger system involved in the GH releasing action by GHRP-6 and related peptides. The modifications in the GH releasing activity by the administration of phloretin, a protein knase-C- inhabitor, and phorbol 12-myristate, 13 acetate (PMA), a protein kinase-C activator was also observed on GH release in juvenile rats. The effects on the GH release was observed after challenging GHRH and peptide 96/257 with phloretin or PMA. Blood collection, serum separation and storage was done as above as mentioned above.

CONCLUSION

Radioactive tracers are useful for radioimmunoassay because of the ease and accuracy with which radioactive ligands are measured. The isotopes which are most commonly used in the receptor measurement studies are Tritium (^{3}H), Carbon (^{14}C) and Lodine-125 (^{125}I). ^{125}I is ideally used for labeling peptides and proteins due to its high specific activity, easy detection and longer half-life (60 days). Higher energy gamma emission does not require additional phosphors e.g. P.P.O. and POPOP and sophisticated instrumentation.