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The Hamster's (*Mesocricetus auratus)* Progesterone Receptors Levels According to the Menstrual Cycle.

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ABSTRACT

The progesterone receptor is a protein that allows for the binding of progesterone in the uterus. The hamster was used because the size is small and the menstrual cycle is fast for high turnover in progesterone levels. The hamster has a menstrual cycle of four days exactly. There is little study on the correlation with the levels of progesterone receptors and the day of the hamster's cycle. This study was to see if the progesterone receptor level does or doesn't change according to the menstrual cycle. This progesterone receptor study went through several processes to finalize the results. The uterus was extracted, separated into components, separated by gel electrophoresis, Western Blotting which includes the following; transferred to cellulose membrane, blocking, attachment of primary and secondary antibodies, and color development. The progesterone receptor level didn't change over the four days of the cycle.

INTRODUCTION

Progesterone is a steroid hormone that regulates a variety of cellular processes in target tissues by altering the rates of specific gene transcription. Progesterone is the only steroid hormone that is essential for the establishment of pregnancy in all mammalian species studied, but its mechanisms of action on target cells are poorly understood. Progesterone influences the growth, development and function of female reproductive tissues by interacting with an intracellular receptor that is a hormonal regulated transcription factor. (Schimdt-Nielson et. al. 1990) Working with the progesterone receptor could lead to several different results in cellular and molecular approaches. The research also could be utilized to determine the effects of over expression and disruption of ovarian progesterone receptors on female reproductive physiology. This study could enhance understanding of the regulation and function of ovarian progesterone receptors and provide a framework for determining whether alterations in progesterone receptor function might participate in reproductive diseases or dysfunction. Cells that produce hormones represent only a limited number of cell types. By contrast, practically all cells of the body are target cells. Since the body cells are exposed to equal concentrations of hormones, receptors found in the plasma membrane are quite specific in that they recognize only certain hormones. Because of the specific complementary structure of the hormone and the receptor, only certain hormones bind to certain receptors in the cell. It is for this reason that a hormone influences some target cells but not others.

Once a hormone is bound to a specific receptor, the combination activates a chain of events within the target cell in which the physiological effects of the hormone are expressed. Receptors, like other cell proteins, are constantly synthesized and degraded and change in concentration and affinity in response to changes within the body. There are generally 2,000 to 100,000 receptors per target cell. (Freifelder et. al. 1987)

Progesterone, the hormone of maturation, works with estrogen to prepare the endometrium for implantation of a fertilized ovum and the mammary glands for milk secretion. Progesterone, like estrogen, is synthesized from cholesterol or acetyl coenzyme A in the ovaries. Following ovulation, the corpus luteum develops. The corpus luteum then secretes increasing quantities of estrogen and progesterone. Progesterone is responsible for preparing the endometrium to receive a fertilized ovum. The functionally dominant ovarian hormone during this phase is progesterone. The decreased secretion of progesterone and estrogen by the degenerating corpus luteum then initiates another menstrual period. By looking at the relative concentrations of the progesterone receptors in accordance with what phase of the menstrual cycle the hamsters were in, it should be possible to see if there is any correlation between the phase of the menstrual cycle and the concentration of progesterone receptors on the target cells.

MATERIALS AND METHODS

The mechanisms and actions of the research done on the hamsters progesterone receptors study are not some very studied processes. Protocols and procedures were not available for reference, so the following procedures were designed during research of studying these hamsters.

Using surgical techniques by Dr. Bobb, the uterus was extracted and put into the dissolving solution to help clean up the uterus and get some of the blood off of the uterus. This is repeated twice and then put on ice. The uterus then is homogenized into and fine mucous so that there is just a liquid form of the uterus left.

The following components will be analyzed. 1)cytosol (cy), 2) organelle/microsome (om), 3) nuclear extract (ne)

Remove tissue from a hamster, put in saline (PBS, pH 7.4) at least 3 volumes. PBS (dissolve 8 g of NaCl, 0.2 g of KU, 1.44 g of Na2 hPO4, and 0.24 g KH2PO4 in 800 ml of distilled H20. Adjust the pH to 7.4 with HCL. Add H2O to 1 liter.) Allow to soak with occasional agitation for at least 5 min. Repeat a soak in PBS at least two more times (for steps two and three keep on ice.) Weigh out 3 grams of a uterus. Transfer tissues to cold TE (pH 8.5)-5ml, homogenize with a hand-held homogenizer, 1 min. on ice. (TE-10 mM Tris, 1 Mm EDTA) Transfer to a conical centrifuge tube, bring to 10 ml with cold TE. Rinse homogenizing containers with 5 mil TE and add to a conical centrifuge tube for a total volume of 15 ml. Spin 10 min at @ 2500 rpm in a 55-34 rotor. Transfer supernatant to fresh tube, fill to 15 ml with cold Te. Spin supernatant at @ 1500 rpm for 15 min. Resuspend pellet form to 15 ml with TE+300 mM HCL. Allow to incubate for 15 min. (Suspension-TE+300mM HCL (100 ml), 1 ml 1M Tris, 200 ul .5 M EDTA, 12 ml 2.5 M HCL, 86.8 ml H2O) Spin at @ 15,000 rpm for 15 min. Spin supernatant form again at @ 15,ooo rpm for 15 min. (be sure to bring volume to 15 ml with cold TE before spinning) Supernatant from it is the cytosol, bring to 15 ml. Resuspend pellet form together, bring to 15 ml , which is OM. Supernatant form , bring to 15 ml and it is the nuclear extract. Resuspend pellet form, bring to 15 ml and that is the nuclear pellet. Aliquot each fraction into smaller containers. Freeze samples. Add 4X sample buffer to samples at a concentration of 1 volume buffer: 3 volume samples. Samples are now ready for polyacrylemide gel electrophoresis.

The solutions were then denatured by heating the solutions up in boiling water for about 3 minutes. The fractions will be read by gel electrophoresis techniques used in the Molecular Cloning laboratory manual found on page 18.47-18.55. The gel will be stained with the coomassie brilliant blue dye. Once there is confirmed protein separation a Western Blot can be done. A Western Blot will be done on the next gel and the results will be analyzed. Western blotting, in which proteins are electrophoretically separated and transferred to a paper to which the protein is covalently linked. Western blots are used to detect DNA-binding proteins. Equilibrate gel in electro transfer buffer for 1hour. Soak nitrocellulose membrane in methanol. Wet all components of gel sandwich in electro transfer buffer. The blot is run at 400 milliamps for 1.5 hours and rinsed with TTBS 5 times. Block with blocking solution (dry milk) (.7g/ 15 ml H20) for 45 minutes. Wash with TTBS 5 times. The primary antibody (progesterone receptor 15 ul/15 ml TTBS) is added for 4.5 hours and washed again with TTBS five times. The secondary antibody (Igg 6ul ExtraAvidin and 12ul Anit-Mouse IgG in 24ml TTBS) is added for 2 hours. Wash with TTBS again 5 times and apply color development (1tablet in 10 ml H2O) for 5 minutes.

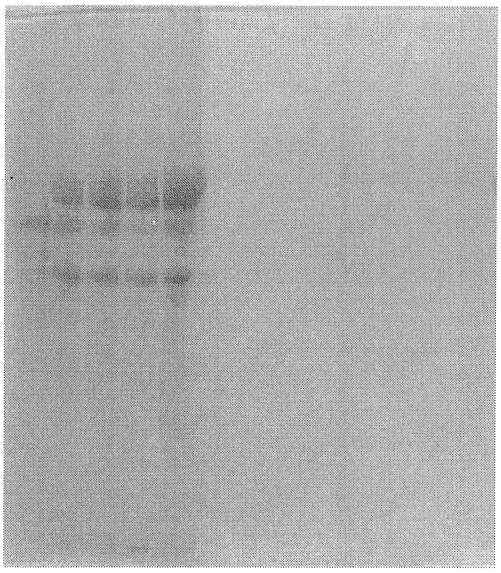

Figure 1: Acrylamide Gel Protein Separation.

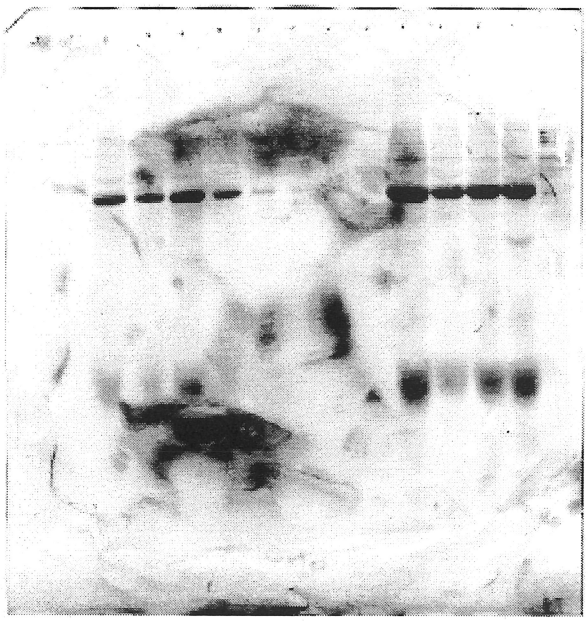

Figure 2: Western Blot Primary and Secondary binding.

RESULTS

Figure 1 is the first gel demonstrating the separation of the components. Figure 2 is the Western Blot results showing the transfer of the molecules to the nitrocellulose paper. Figure 3 is a Western Blot result of using just the secondary antibody to look for any variation. Figure 3 shows 4 light colored bands in the top right hand corner of the blot.

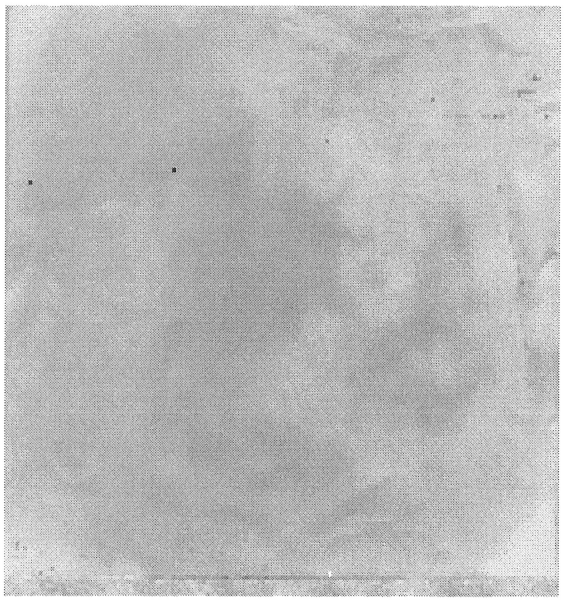

Figure 3: The control Western Blot Secondary binding.

DISCUSSION

The gel electrophoresis had good separation and the Western Blot showed strong attachment in the cytosol and nuclear extract columns all four days of the menstrual cycle. The control Western Blot confirmed the progesterone receptor binding by showing a difference in the primary western blot and the control western blot. The organelle microsomal fractions of each day didn't show any signs of progesterone receptor levels. This is not surprising since the attachment of the progesterone receptor is in the cytosol and transported into the nucleus. The levels of the progesterone receptors didn't change over the four days. Displayed in Figure 2 it shows that the level maintained a close balance of concentration of progesterone receptors.

ACKNOWLEDGEMENTS

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