

Steroid Bioassay Laboratory

Introduction

Steroids are a family of chemical substances comprising many hormones, vitamins (vit. D) and drugs (digitalis). Each contains the tetacyclic-cyclopenta[a]phenanthrene skeleton. Steroid hormones are produced in the adrenals, ovaries and testes and have varying biological effects.

The first of the steroid hormones to be discussed is testosterone. Testosterone causes general anabolic effects (increased weight) and tissues such as prostate and seminal vesicles are especially sensitive to the action of testosterone. Testosterone is also very important in maintaining fertility.

The second hormone, estrogen, is the female sex hormone important in maintaining female sex characteristics and fertility. Estradiol is the primary circulating estrogen in non-pregnant females. Estradiol levels fluctuate throughout the menstrual (humans) and estrous cycles (rats). Uterus weight and vaginal cytology can be used to assess circulating estradiol levels.

In this lab, we will remove the testes or ovaries from rats, and determine how this affects steroid-responsive and control tissues (by measuring their weight) in the presence or absence of steroid replacement therapy.

EXPERIMENTAL PROCEDURES TO BE DEMONSTRATED BY INSTRUCTOR:

Orchidectomy (surgical removal of the testes)

1. Anesthetize the male rat using isoflurane anesthesia. Place rat in induction chamber, set the gas vaporizer on '5'; monitor rat and remove from chamber when it is immobile.
2. Immediately clip the hair from the scrotum and surrounding area; position animal on back and with nose in nose cone as demonstrated by Instructor.
3. Make certain animal is in surgical plane of anesthesia (set vaporizer to '3').
4. Disinfect the scrotum with Betadine.
5. Make an incision through the skin of the scrotum using sterile scalpel.
6. Make second incision through the transparent tunica vaginalis.
7. Gently manipulate first testis through opening; place ligature around tunica intima and spermatic cord - must be tight to prevent bleeding!
8. Cut the testis free by making cut distal to the ligature (save this tissue for the microscope!).
9. Repeat steps 6-8 for second testis.
10. Staple the scrotal skin closed.
11. To implant pellet: shave hair from between shoulder blades, make small opening (pellet-sized) with sharp scissors, clear subcutaneous connective tissue by blunt dissection, insert pellet, and close incision with a single wound clip.
12. Weigh the animal and record its weight.
13. Keep the animal warm and constantly monitor its condition until it awakens.

Ovariectomy (surgical removal of the ovaries)

1. Anesthetize the animal as described above.
2. Immediately clip the hair from surgical area; position rat on its stomach.
3. Disinfect the surgical site with Betadine.
4. Using disinfected, sharp scissors, make a 1-1.5cm cut in the skin midway between last rib and the knee in the papalumar fossa.
5. Make a second cut through the muscle layer into the peritoneal cavity.
6. You should be able to see the fat pad containing the ovary through the surgical opening. The ovary resembles a translucent cluster of grape-like structures.
7. Carefully withdraw the fat pad through the opening; isolate the ovary and tie it off with a disinfected silk ligature.
8. Cut the ovary off, leaving the ligature on the tip of the uterus (save this tissue for the microscope!).
9. Carefully return the ligated uterus into the body cavity.
10. Close the peritoneum and muscle layer with a suture or two as needed, and close the skin with wound clips.
11. Repeat steps 3-10 for the other ovary.
12. Implant the pellet as described above.
14. Weigh the animal and record its weight.
15. Keep the animal warm and constantly monitor its condition until it awakens.

Uses for male and female gonadal tissue:

1. Under the dissecting microscope, examine the testis and structures. Can you find the epididymis and seminiferous tubules? Insert a needle into different areas of the epididymis, collect sperm into a drop of saline, and examine their motility under the microscope. Collect sperm from the testis by making a small incision and collecting fluid on microscope slide; how are these sperm different from epididymal sperm?
2. Examine the ovaries under the microscope. Carefully cut off the oviducts, place on microscope slide, place a second microscope slide on top of oviducts and squeeze slides together to flatten oviducts. Tape slides together. Look at slide under microscope and see if there are ovulated follicles in the oviducts. With the ovaries, can you identify follicles and corpora lutea?

AFTER 14 DAYS YOU WILL EUTHANIZE THE ANIMAL AND PERFORM A NECROPSY.

Necropsy

1. Euthanize the animal with an overdose of pentobarbital (200mg/kg body weight, given intraperitoneally).
2. Record the animal's weight.
3. Remove the following tissues (as demonstrated by Instructor) and remove all fat and connective tissue; record each tissue's weight.

Male animals:

Seminal vesicles
Prostate gland
Spleen
Adrenal glands
Pituitary gland

Female animals:

Uterus
Spleen
Adrenal glands
Pituitary gland
Vaginal cells (for microscopic analysis only)

Data from all of the lab sections will be compiled and provided to student at next lab meeting. You will be expected to average the results (undergraduates: perform statistics for extra credit; graduates must perform statistics) and prepare a 2-3 page report briefly describing the experiment and discussing the experimental results. Return your report to your TA by Fri. November 17 (NO exceptions); the report will be graded by Dr. Murphy.