Histol Histopath (1987) 2: 143-145

Histology and Histopathology

Autoradiographic localization of estrogen target cells in the spinal cord of the armadillo and baboon: a comparative study

Frank J. Weaker and Peter J. Sheridan
Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, Texas, USA

Summary. The uptake and retention of radiolabeled estradiol by the spinal cord were examined in the baboon and the armadillo and compared to previous observations in the rat. Four females of each species were injected intracardially with 1.0-1.4 μg/kg body weight of ^{3}H-estradiol and two females, one baboon and one armadillo, were injected with both labeled and 100-140 μg/kg body weight of unlabeled estradiol. One hour after the injections, the animals were killed and segments from the cervical, thoracic, lumbar and sacral cord were removed and processed for autoradiography. In the armadillo, labeling of neuronal nuclei were noted in laminae I & II and in alpha motor neurons. In addition, nuclei of the ependymal cells of the ventral portion of the central canal in the cervical cord concentrated radioactivity. In contrast, the baboon demonstrated only sporadic labeling of neurons in lamina II in all levels of the spinal cord. The comparison of our observations with that of the rat suggest that estrogen mediated sensations are probably coordinated at higher brain centers in the primate as opposed to the more primitive mammals.

Key words: Autoradiography - Receptors - Alpha motor neurons - Ependyma

Introduction

It is well known that gonadal steroids are involved in the sexual differentiation of the central nervous system, and that changing levels of these hormones affect specific neurons in the brain and spinal cord that control reproductive function (Gorski, 1971; Breedlove and Arnold, 1983). Furthermore, studies have indicated that some sexual responses are mediated in the spinal cord (Hart, 1978). Previous autoradiographic studies in the spinal cord of the rat demonstrate the localization of estradiol in the sensory areas, laminae I and II, and androgens in the motor system. It has been postulated that estrogen is involved in the modulation of sensory information (Keefer et al., 1973) and that androgen is related to motor function (Stumpf and Sar, 1979). However, in a previous study of the spinal cord of the rhesus monkey (Sheridan and Weaker, 1981), we demonstrated nuclear uptake of dihydrotestosterone in lamina II and in the intermediomedial and intermediolateral cell columns of lamina VII as well as in the alpha motor neurons of lamina IX in the ventral horn. The widespread distribution of androgen receptors in both motor and sensory portions of the spinal cord suggests a role for this steroid in both sexual sensations and reflexes as well as nociception.

The following comparative study demonstrates differences in the localization of estradiol in the spinal cord of the baboon and the armadillo, a primitive mammal, and compares the distribution of the receptor sites to that found in the rat.

Materials and methods

Tissues for this study were obtained from five adult female nine-banded armadillos, *Dasypus novemcinctus,* and five adult female baboons, *Papio cynocephalus.* The female baboons were sexually mature and cycling normally; their weight ranged between 15.5-19.7kg. Three days prior to the injection of the tritiated steroid, both gonads and the right adrenal gland were removed from each animal under ketamine and halothane or fluothane anesthesia with aseptics surgical procedures. Two days later, the left adrenal gland was removed under similar conditions. Shortly after the second operation, each animal received 100mg of prednisolone sodium succinate to help prevent shock. On the day of the experiment, each animal was anesthetized with ketamine

Offprint requests to: Dr. Frank J. Weaker, Department of Cellular and Structural Biology, 7703 Floyd Curl Drive, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284, USA.

and intravenously injected (femoral vein) with 1 µg/kg body weight of [2,4,6,7,16,17 ^{3}H] - estradiol-17ß (^{3}H-E_2) (136 Ci/mole). One female which served as a control, was injected with 100 µg/kg body weight of unlabeled estradiol in addition to the ^{3}H-E_2. One hour after injection each animal, still under ketamine anesthesia, was rapidly exsanguinated through a femoral venous catheter, and the vascular system was perfused with chilled Ringer's solution through a femoral arterial catheter. As each animal was exsanguinated and perfused, the body was also packed in ice to accelerate chilling. When the perfusated became clear, usually after 20-30 min., the spinal cord was removed for dissection. Because of the value of these animals, several organs systems were removed for parallel studies.

The armadillos were caught in the wild in southern Texas during the month of February when the animals are reproductively quiescent, and thusly were not castrated. A dose of 1.4 µg/kg body weight of [2,4,6,7 - ^{3}H) estradiol (101 Ci/mmole) was injected into the left ventricle of each 4 armadillos. For a control, one animal was injected with the same dose of ^{3}H-steroid plus 140 ug/kg body weight of unlabeled estradiol. One hour after injection, the animals were perfused with physiological saline via the left ventricle, and the spinal cord removed for further dissection.

The autoradiographic procedure is described in detail elsewhere (Stumpf, 1971; Ison and Sheridan, 1981). Briefly, segments of the cervical, thoracic, lumbar and sacral cords of both species were placed on brass tissue holders and simultaneously frozen and mounted by immersion in liquified propane chilled to approximately -180°C. Four micrometer thick sections were cut at -30°C nife temperature using a Wide-Range Cryostat. In the darkroom, sections were then mounted directly on Kodak NTB-2 emulsion-coated slides by bringing the slides into brief contact with the upper surface of the knife so that the sections adhered to the emulsion by melting from the heat of the slides. The slides were exposed at -15°C for a period of 3-6 months, then developed for 1 min at 18°C in Kodak D19 developer, rinsed, and fixed for 5 min in Kodak fixer, rinsed, and stained with hematoxylin and eosin. A cell was considered labeled if the number of grains of the nucleus exceeded three times the silver grain count in the adjacent extracellular space and cytoplasm. All photomicrographs were taken on an Olympus light microscope.

Results

Nuclear localization of ^{3}H-E_2 was observed in the gray matter of the spinal cord of both species; however the uptake of estradiol was more noticeable in the armadillo than in the baboon. No localization was found in the white matter.

Neurons in laminae I and II of the dorsal horn in the armadillo (Fig.1) were radiolabeled at all levels of the cord studied. In addition, ependyma cells lining the central canal in the cervical cord contained silver grains.

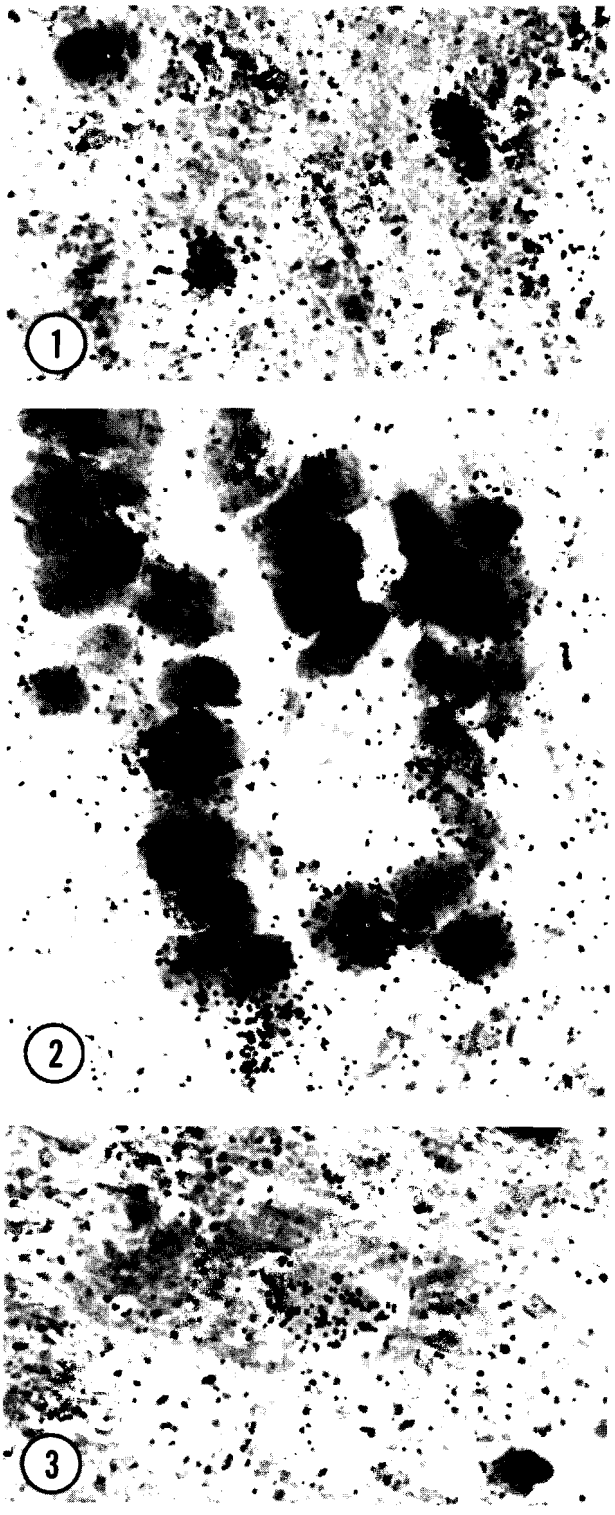

Fig. 1-3. Autoradiograms of spinal cords from armadillos injected with ^{3}H-estradiol. Nuclei of neurons in laminae I and II (Fig. 1), ependymal cells of the central canal (Fig. 2) and alpha motor neurons (Fig. 3) concentrated the radiolabeled ligand. X1,050

However, the distribution of radioactivity was unusual in that only the cells on the ventral aspect of the canal were labeled (Fig. 2). Another unusual feature of the armadillo was the radiolabeling of the alpha motor neurons which was particularly conspicuous in the lumbar segments (Fig. 3). Labeling in the spinal cord of the baboon was very sporadic and limited to isolated cells (no more than one per section) in lamina II of all levels of the cord (Fig. 4). The ependyma and alpha motor neurons were not labeled.

In control animals (both armadillo and baboon) injected with ^3H-E_2 plus unlabeled estradiol, nuclear labeling in all the aforementioned cells types was either absent or significantly diminished (Fig. 5).

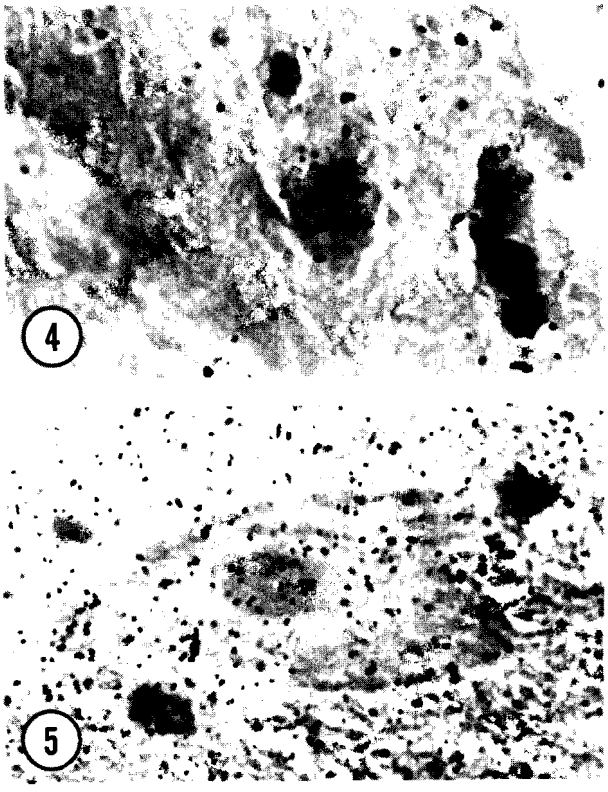

Fig. 4. Sporadic labeling of neurons in lamina II were observed in the spinal cords of baboon injected with ^3H-estradiol. X1,050
Fig. 5. Alpha motor neuron of the spinal cord of the armadillo simultaneously injected with labeled plus unlabeled hormone demonstrating a lack of nuclear concentration of silver grains. X1,050

Discussion

Although the autoradiographic localization of estrogen in the brain has been studied in more species than with any other steroid (see review, Sheridan, 1984), the uptake and retention of estrogen in the spinal cord using this technique has been limited to the rat (Keefer et al., 1973). These investigators observed that sensory areas in the spinal cord, laminae I and II, contained neurons radiolabeled for E_2.

In the armadillo, alpha motor neurons and the ependyma concentrated ^3H-E_2 in addition to laminae I and II as reported in the rat (Keefer et al., 1973). Although the function of the alpha motor neuron was not ascertained in our study, the localization of ^3H-E_2 suggests that estrogen is involved in motor function as well as in sex-related sensations that have been postulated to exist in the rat. Stumpf and Sar (1977) have demonstrated the presence of several ventricular recess organs which concentrate gonadal steroids. It is possible that the ependyma cells labeled in the cervical cord of the armadillo may be related to these organs.

The sporadic labeling of cells in the baboon spinal cord was surprising in that we expected sensory areas to concentrate estrogen because of the data from the armadillo and the rat. Additionally, several sensory systems in the brains of the very same animals used in this study demonstrated nuclear localization of radiolabeled estrogen (unpublished observations). These observations suggest that estrogen mediated sensations are probably coordinated at higher brain centers in the primate as opposed to the more primitive mammals.

References

Breedlove S.M. and Arnold A.P. (1983). Hormonal control of a developing neuromuscular system. J. Neurosci. 3, 424-432.

Gorski R.A. (1971). Gonadal hormones and the perinatal development of neuroendocrine function. In: Frontiers in Neuroendocrinology. Martini L. and Ganong W.F. (eds). Oxford Universitry Press. New York. pp 237-290.

Hart B.L. (1978). Hormones, spinal reflexes, and sexual behavior. In: Biological Determinants of sexual behavior. Hutchison J.B. (ed). John Wiley & Sons, New York. pp 319-348.

Ison E.J. and Sheridan P.J. (1981). Autoradiography of diffusable substances - A practical approach. Am. J. Med. Technol. 47, 38-42.

Keefer D.A., Stumpf W.E. and Sar M. (1973). Estrogen-topographical localization of estrogen-concentrating cells in the rat spinal cord following ^3H-estradiol administration. Proc. Soc. Exp. Biol. Med. 143, 414-417.

Sheridan P.J. (1984). Autoradiographic localization of steroid receptors in the brain. Clin. Neuropharmacol. 7, 281-295.

Sheridan P.J. and Weaker F.J. (1981). The primate spinal cord is a target for gonadal steroids. J. Neuropathol. Exp. Neurol. 40, 477-453.

Stumpf W.E. (1971). Autoradiographic techniques for localization of hormones and drugs at the cellular and subcellular level. Acta Endocrinol. [Suppl] (Copenh) 153, 205-211.

Stumpf W.E. and Sar M. (1977). Steroid hormone target cells in the periventricular brain: relationship to peptide hormone producing cells. Fed. Proced. 36, 1973-1977.

Stumpf W.E. and Sar M. (1979). Steroid hormone target cells in the extrahypothalamic brainstem and cervical spinal cord: Neuroendocrine significance. J. Steroid. Biochem. 11, 801-807.

Accepted October 14, 1986