Autoradiographic Identification of Estradiol-concentrating Cells in the Spinal Cord of the Female Rat*


The Rockefeller University, 66th Street and York Avenue, New York, NY 10021, USA

Summary. The topography and number of estradiol (E)-concentrating cells in the lower lumbar and sacral segments of the spinal cord of the female rat have been examined by the steroid autoradiography method. A nuclear-saturating dose of E was administered by intravenous infusion, which kept blood estrogen at or above proestrus levels for 3.5–4 h, much longer than usual for steroid receptor studies. The cord segments selected for examination are known to receive somatosensory information relevant for estrogen-dependent behavior, and to contain some of the motoneurons for epaxial muscles responsible for this behavior.

Small numbers of E-concentrating cells were found in the dorsal portion of the gray matter of L₄, L₅, L₆ and the sacral segments. These cells were found in lamina II, in the midline region which includes lamina X, and the medial portions of laminae III, IV, and V when they cross in the midline. E-concentrating cells were also found in the lateral portions of laminae III, IV, and V, and in lamina VII. Virtually no E-concentrating cells were found in the ventral portion of the gray matter or in the white matter. The spinal cord had few E-concentrating cells compared to the hypothalamus.

Key words: Estradiol – Autoradiography – Spinal cord

Introduction

The presence of estradiol-concentrating cells in the hypothalamus and limbic system of the rat brain has been documented (Pfaff 1968; Pfaff and Keiner 1973). Large numbers of estradiol-concentrating cells have been found in the medial preoptic area, the medial anterior hypothalamus, the ventromedial, the ventral premammillary and the arcuate nuclei. Estradiol-concentrating cells have also been found in the septum, the medial nucleus of the amygdala, and in the mesencephalic central gray. There is a large number of these cells, and they are intensely labelled after estradiol administration, evidence of a considerable hormone concentrating capacity.

In fact, examination of a large number of vertebrates has demonstrated that there is a stable, vertebrate-wide pattern for estradiol-concentrating cells (Morrell and Pfaff 1978), with large numbers of hormone-concentrating cells found in these areas in all the other species examined.

There has been less exploration of estradiol-concentrating cells in non-hypothalamic and non-limbic structures, although initial studies (Pfaff 1968) demonstrated small numbers of some estradiol-concentrating cells in the pons, medulla and cervical spinal cord. It appeared reasonable to examine more closely the spinal cord for the presence and pattern of estradiol-concentrating cells. Would we uncover more information about estradiol-concentrating cells if the conditions for hormone binding could be maximized, and the examination of the spinal cord focused on a portion which was relevant for a hormone-dependent behavior?

The lumbar region of the rat spinal cord, specifically the lower lumbar region, has been demonstrated by two different experimental approaches to be particularly relevant for the display of lordosis, a female sexual behavior which depends on estrogen. It has been demonstrated that the somatosensory input from the skin on the perineum and the tail base of the female is particularly important for the initiation of lordosis behavior. This cutaneous information enters the cord via dorsal roots L₅ and L₆ (Kow and
In addition, some of the motor neurons which control lateral longissimus and the lumbar transverso-spinalis muscles, trunk muscles which execute the lordosis reflex, have been demonstrated to be located in the lower lumbar spinal cord (Brink et al. 1979).

This study was undertaken to examine segments of the spinal cord which are particularly relevant for the estrogen-dependent behavior, lordosis, for the presence of estradiol-concentrating cells. Methodological steps were taken to maximize demonstration of all estrogen-concentrating cells. The spinal cord autoradiograms examined in the present study were compared to hypothalamic autoradiograms also prepared from the same animals.

Materials and Methods

Animals

Eleven healthy female rats (nine Sprague-Dawley, Hormone Assay; and two Long-Evans, Charles River), body weight 250-290 g, were adrenalectomized and ovariectomized 1-4 weeks before isotope administration. Two were simply ovariectomized. Adrenalectomized animals were given 2% sucrose and 0.9% saline solution to drink in addition to water.

Infusions: $^3$H-Estradiol

The infusion was designed to keep the level of estradiol to which the brain was exposed at or above proestrus level for a total of 3-4 h. This gave the brain and spinal cord a maximum chance at estradiol uptake. The animals were given a nuclear saturating dose (McEwen, pers. commun.) of $^3$H-2,4,6,7 estradiol 0.8 µg/250 g body weight (S.A. 80–102 Ci/mmol New England Nuclear; absolute ethanol solution). The ethanol was evaporated so that no more than 0.1 ml was given per animal. The infused solution was tritiated estradiol in 0.1 ml absolute alcohol; 1 ml rat serum (from another rat); 0.4 ml heparin (5,000 units/ml); total volume 1.5 ml. Rat serum increased solubility of the estradiol.

Four animals were infused with 0.8 µg estradiol/250 g b.w. The animals were anesthetized with urethane (7 ml/kg; 20% solution), given 0.5 ml atropine, tracheotomized, and catheters were placed into jugular (for blood samples) and saphenous (for infusion) veins. For infusion, a 30 gauge needle fitted with polyethylene tubing (PE-10) was placed in the vein; the other end of the tubing was fitted onto another 30 gauge needle, on a 2.5 ml syringe in a Harvard infusion pump apparatus. The animal was infused with the radioactive estradiol (with or without cold competitor or anti-estrogen) for a period of 2 h. Two hours post-infusion, the animal was killed by guillotine, and the spinal cord and brain quickly removed from their bony coverings.

Two animals were given the same hormone dose injected i.p. Half the dose was given; after 0.5 h the remaining half was given and the animal killed 2 h later. Results were compared to results from animals given isotope by infusion and no significant differences were found. The data from the six animals were therefore considered together.

The chemical identity of the isotope was assessed by using standard thin layer chromatography. In every case, the majority of the tritium was travelling in the estradiol peak.

Fig. 1. Diagrams of representative transverse sections through four segments of the spinal cord. Each black dot represents one estradiol-concentrating cell; all such cells present in each section are represented. Exposure time of autoradiograms: 12 months Rexed laminae II, III, IV, V, VII, IX, X are indicated. Total number of labelled cells: L4 = 24; L5 = 17; L6 = 7; S1 = 5