Sexual Differentiation of the Spinal Nucleus of Bulbo-Cavernosus Muscle (Onuf's Nucleus)

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Abstract

Background: The brain seems to be inherently feminine. Masculine characteristics of the structure and functions are imposed on developing CNS by the action of testicular hormones during a critical embryonic period. Testosterone is a prohoromone responsible for sexually dimorphic characteristics of the brain. A number of structural sex differences in the human brain have been reported, but their functional significance are less well established. Brain function in men appears to be more lateralized than women and this may be the reason that women are more likely than men to recover speech, after a stroke that damages cortical speech areas.

Objective: this study examines the effects of testosterone on sexually dimorphic nucleus of the rat spinal cord i.e. the spinal nucleus of the bulbocavernosus muscle, which innervates two sexually dimorphic, androgen-sensitive perineal muscles that are active during copulation, the bulbocavernosus and levator ani.

Methods: the study was performed on litters of albino rats. Their pregnant mothers received on embryonic days (E16- E20) subcutaneous injection of 2mg of testosterone propionate. The spinal nucleus of bulbocavernosus was examined in control group, female litters with and without prenatal testosterone injection.

Results: It was demonstrated that perinatal injection of testosterone prevented the involution of spinal nucleus of bulbocavernosus in the female rats.

Conclusions: It is concluded that androgens have a sparing effect on the motor neurons of this nucleus that prevent the developmental cell death of neuronal cells. The mechanism of the effects of androgen are discussed, whether it is through direct effect of androgen on motor neurons themselves, or indirect effect as a result of the androgen action on the target androgen-sensitive perineal muscles.

Key words: Onuf's nucleus, testosterone, sexual differentiation

Introduction:

Sexual differentiation of the brain has been conclusively demonstrated in many mammalian species. The brain seems to be inherently feminine. Masculine characteristics of the structure and function are imposed on the developing CNS by the action of testicular hormones during a critical period. Sexual differentiation takes place through the effect of Y chromosome Testis Determining Factor (TDF) gene that leads to the development of testis and production of testosterone and its metabolites. Testosterone is a pro-hormone for dihydrotestosterone and estradiol that are responsible for sexually dimorphic characteristic of the brain [(1)].

One of the most evident sexual dimorphic features of the central nervous system are those related to the innervation of perineal muscles. The bulbocavernosus muscles surround the base of the penis and have an important role in penile erection and help to eject urine. Both male and female have bulbocavernosus muscle. In female, it surrounds the opening of the vagina and serves to constrict it slightly.

The alpha motor neurons that are responsible for the innervation of the bulbocavernosus muscle are located in the lumbar spinal cord, and in particular, at the fifth lumbar segment of the spinal cord. These motor neurons form a distinct cluster of large neurons (magnocellular) that is called the spinal nucleus of bulbocavernosus. In a transverse section of the lumbar spinal cord, this nucleus is localized at the margin of the anterior horn of gray matter of the spinal cord near the anterior white funiculus [(2)].

The motor neurons pool controlling the bulbocavernosus muscle in rat, cat, dog and human is called Onuf's nucleus. It is located in the sacral part of spinal cord in human. It is a sexually dimorphic nucleus [(3)].

The aim of this study is to examine the effects of testosterone on a sexually dimorphic structure in the rat spinal cord. The spinal nucleus of the bulbocavernosus innervates two sexually dimorphic, androgen-sensitive perineal muscles that are active during copulation, the bulbocavernosus and levator ani. Adult female rats have fewer and smaller motor neurons in this spinal nucleus, than do males. The spinal nucleus of the bulbocavernosus muscle rapidly involutes, almost entirely, within the first few days after birth [(4)].

Materials and Methods:

This study was performed on female litters of albino rats (Rattus rattus norvegicus albinus). Male and female rats were kept in mating cages, provided with water and standard rat chow ad libitum at 25°C ambient temperature with equal light and dark diurnal variations.

The morning of finding vaginal plug in the dripping tray was considered as E1 day of embryonic development and the female was transferred to separate cage.

On days E16-E20 each pregnant rat was injected subcutaneously with 2mg of testosterone propionate (gsk) dissolved in sesame oil.

Some of the litters were borne normally on E22, but approximately 2/3 of the pregnant rats failed to deliver the pups by E23 (an unexpected side effect of testosterone injection). In that case, the pregnant rats were anesthetized with ether, and the pups were delivered by caesarian section and fostered to another lactating female rat.

After birth, the female litters were identified by observing the distance between the urethral orifice and the anus. The female litters were given 1mg testosterone propionate in sesame oil subcutaneously on post natal days one and three. This treated group of female litters included 20 litters. It was compared with identical group of another 20 female litters that received no testosterone injection. Five male litters were studied as a reference.

At postnatal day 7, the litters were sacrificed by lethal dose of pentobarbital. Spinal cords were removed and fixed in

4% Para formaldehyde for overnight. The following day, the lumbo-sacral segments of the spinal cord were identified and processed for paraffin embedding and sectioned in transverse plane. The sections were stained with hematoxylin and eosin and examined.

Results:

The examination of transverse sections of the spinal cord revealed the cluster of motor neurons constituting the bulbocavernosus nucleus. In male litters, this nucleus was demonstrated in the fifth lumbar segment of the spinal cord, located on the ventral aspect of the anterior horn of the gray matter. It is located at the region bordered by the anterior white commissure of the spinal cord, the central canal and the base of the anterior horn of gray matter. The nucleus was virtually absent in female control litters and no magnocellular motor neurons can be identified at that region. Examining the female litters that had received injections of testosterone propionate during their late pre-natal life and early post natal days (the study group), it was possible to identify the cluster of motor neurons forming the bulbocavernosus spinal nucleus persisting in the lumbar region of the spinal cord at post natal day 7. It was identical with the configuration demonstrated in the male litters of same age that served as reference (Figure 1).Other motor neurons demonstrated on the same spinal cord sections have not been affected, which indicates that the testosterone does not have a non-specific action on motor neurons.

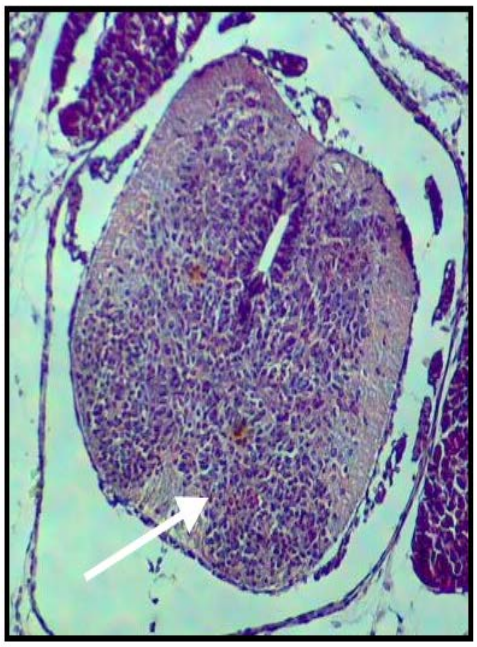

Figure 1: Transverse section of the spinal cord at the level of fifth lumbar segment. Fro female rat litter receiving testosterone injection. Obtained at seventh postnatal day. The w arrow mark well developed, magnocellular bulbocavernosus nucleus analogous to tha male litters. H&E.

Discussion:

This study was carried out on the spinal nucleus of the bulbocavernosus in the rat. It is a nucleus that undergoes degeneration and involution rapidly in female litters during the first few days of life.

This study demonstrated that the peri-natal injection of testosterone (during late pre-natal and early post-natal life) prevents the involution of the motor neurons of this nucleus. Such testosterone injections in newborn female rats maintain the male pattern of this sexually dimorphic nucleus of the spinal cord. During normal male pattern of development, the testes produce a perinatal surge of androgen, which apparently acts to prevent degeneration of the spinal nucleus of the bulbocavernosus muscle, and subsequently prevents the motor neuron death that occurs in females at this time [(5)].

The mechanism of action of these effects of androgens is suggested to be through a direct effect of androgen on the motor neurons of this nucleus. It was found that in adult male rats, the spinal nucleus of bulbocavernosus motor neurons shrink after castration and are restored in size after androgen treatment [(6)]. Moreover, and in support of this view, it was demonstrated that androgens in adulthood regulate the morphology of the motor neurons of the spinal nucleus of bulbocavernosus. The somata and dendrites of the motor neurons of this nucleus show androgen-sensitivity [(7)]. Another possibility of the mode of action of androgens is through indirect effect, as a result of the androgen action on the

androgen-sensitive perineal muscles. Androgens receptors in striated muscles are particularly high in sexual muscles. The androgen effects on the spinal nucleus of bulbocavernosus are well established through studies on genetically modified rats. Such effect was absent in androgen-insensitive adult rats [8]. It was also demonstrated in rats with mosaic androgen sensitivity [9].The most sexually dimorphic characteristics of the mammalian brain are clustered around the third ventricle, within the anterior pre-optic area of the hypothalamus. In human, there are four clusters of neurons called the interstitial nuclei of the anterior hypothalamus (INAH). INAH-3 shows the greatest sexual dimorphism, being larger in men than women. INAH-1 may be the human analogue to sexually dimorphic nucleus found in the rat, which is 5-8 times larger in male rats[1]. In the cat, dog and human, the Onuf's nucleus contains motor neurons of the bulbocavernosus and ischiocavernosus as well as those of anal and urethral sphincters. Onuf's nucleus is accounted to be homologous to the spinal nucleus of bulbocavernosus of the rat and is sexually dimorphic as well [3]. A number of structural sex differences in human brain and spinal cord have been reported but these structural differences and their functional significance are less well established than those in experimental animals.

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