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Research report

Effects of male and female sex steroids on the development of normal and the transient Froriep's dorsal root ganglia of the chick embryo

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Abstract

Sex steroids can influence developmental processes and support the survival of neurons in the embryonic central nervous system. Recent studies have shown that estrogen receptors are also expressed in the peripheral nervous system, in the dorsal root ganglia (DRG) of chick embryos. However, no studies have examined the effects of sex steroids on development of embryonic DRG. In the present study, 0.2 μg, 1.0 μg, 5.0 μg 10 μg, 20 μg, 25 μg, and 40 μg doses of testosterone or estradiol were delivered to chick embryos at Hamburger and Hamilton stage 18 (E3). The actions of these doses of sex steroids on the development of the C5DRG (fifth cervical ganglion, a "normal" DRG) and C2DRG (a transient ganglion known as a "Froriep's DRG") were then evaluated by quantifying ganglionic volumes, cell number, proliferation, and apoptosis after 1 day of growth to stage 23. We found that both testosterone and estradiol promoted proliferation of cells in both normal DRG and the Froriep's ganglia. By contrast, estradiol significantly increased the number of apoptotic cells, while testosterone strongly inhibited apoptosis. These actions of sex steroids on DRG development were dose-dependent, and C5DRG and C2DRG showed different sensitivities to the applied sex steroids. In addition, the present results demonstrated that specific ER and AR inhibitors (tamoxifen and flutamide) did not influence the effects of 5 μg E2 and 5 μg T on C2 and C5DRG significantly. These results demonstrate that male and female sex steroids can modulate DRG development through an epigenetic mechanism, as had been shown for the central nervous system.

Theme: Development and regeneration
Topic: Hormones and development

Keywords: Sex steroids; Chick embryo; Dorsal root ganglia; Neuron

1. Introduction

Sex steroids are an important class of hormones that have marked biological effects on many kinds of tissues including the nervous system. For example, the female sex steroid estrogen can promote the growth of the hippocampus, midbrain, cortex, pituitary, and spinal cord of the central nervous system (CNS). In addition, it influences cell migration, survival and death, and synaptic plasticity during neuronal development [7]. There has been much less study of the actions of estrogen on the peripheral nervous system (PNS) compared with the CNS. However, recent reports showed that estrogen receptor (ER)-immunopositive neurons are present in the DRG of rat [35], and chick embryo [10]. In addition, exogenous estrogen has been shown to support survival of cultured DRG neurons by up-regulating the expression of the anti-apoptotic molecules Bcl-x [36], NGF receptors, trkA and $p75^{NTR}$ [39] and Calcitonin Gene-Related Peptide (CGRP) [25].

Many studies have shown that androgen receptors are expressed in the nervous system [5,23,41,45]. Studies by

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Melvin et al. showed that the influences of testosterone on the morphology and neurochemistry of the sympathetic hypogastric ganglion appear to be dependent on the time of androgen exposure and testosterone levels [29]. Other investigations found that androgen could increase the number of cells in fetal mouse spinal cord in vitro [20], and regulate dendritic length of motoneurons in adult rats [24]. In addition, androgen can regulate neuron number in lumbosacral DRG and the spinal nucleus of the bulbocavernosus (SNB) [30,32]. A recent study showed that testosterone and 17-β estradiol affect the differentiation but not proliferation of cultured neurons from the cortex of rat embryos [46].

In contrast to the wealth of studies of the intact CNS and peripheral neurons in vitro, to date there is no direct evidence concerning the action of sex steroids on the development of early embryonic dorsal root ganglia (DRG) in vivo. Our previous research [10] demonstrated the presence of estrogen receptors in chick embryo DRG. We thus postulated that estrogen may play important roles in the development of DRG. The present study was performed to investigate the actions of a male and a female sex hormone (testosterone and 17-β estradiol) on the processes of proliferation and apoptosis in early avian DRG, and establish whether these actions of testosterone and 17-β estradiol on DRG are via specific receptors using flutamide (FLU) and tamoxifen (TAM), respectively.

The DRG are a metameric series of structures that develop from neural crest cells within the dorsal somatic mesoderm. A striking element of axial patterning among the DRG is that the ganglia that develop in the most rostral somites (occipital and first cervical) disappear early in embryogenesis and some fundamental difference first arises between Froriep's DRG and other DRG at stages 18–20 [28]. We here quantify proliferation and apoptosis in both the normal fifth cervical DRG (C5DRG) and in the longer lasting Froriep's DRG (C2DRG) because they have been shown to exhibit significant differences in both of these processes [38].

2. Materials and methods

2.1. Chick embryos and treatment with testosterone and 17β-estradiol

Fertilized White leghorn eggs, all of which were 55.0 ± 0.5 g in this study, obtained from the chick house of China Agricultural University were incubated at 37.5 °C for 3 days (stage 18–19, Hamburger and Hamilton, 1951 [18]). The eggs were then windowed under sterile conditions and the shell membrane was reflected. Subsequently, 0.2 μg, 1.0 μg, 5.0 μg, 10 μg, 20 μg, 25 μg, or 40 μg of 17-β estradiol (Sigma) or testosterone (Sigma) dissolved in 50 μl of 1% ethanol in phosphate buffered saline (PBS) was delivered to the surface of the chorioallantoic membrane or into its cavity. For control embryos, 50 μl of 1% ethanol in PBS without steroids was applied. After the windows were sealed with cellotape, the embryos were returned to the incubator for 24 h.

2.2. Treatment with FLU and TAM

In order to determine whether the effects of 17-β estradiol on the DRG cells are through their corresponding receptors, we firstly administrated 10 μg TAM, the specific ER inhibitor, to the chick embryo. After a 30-min incubation, 5 μg E2 was administrated. In another group, only 10 μg TAM but without E2 was applied. After the windows were sealed with cellotape, the embryos were returned to the incubator for 24 h. To determine whether the effects of testosterone on the DRG cells are through the corresponding receptors, FLU, the specific AR inhibitor with T, were supplied to the chick embryos as above.

2.3. Estimation of cell proliferation

In order to estimate the effects of different doses of 17-β estradiol and testosterone on the proliferation of DRG cells, 100 μl of a 1-mM solution of bromodeoxyuridine (BrdU, Boehringer Mannheim) was injected into the amniotic space [16] and the embryos were returned to the incubator for 1 h. The embryos were then sacrificed, staged, and fixed in 4% paraformaldehyde in PBS, embedded in paraffin, after which 5-μm serial sections were cut and mounted using conventional methods. The sections were immunolabeled as described previously [2]. Briefly, the sections were dewaxed and rehydrated. Subsequently, DNA was denatured in 2 M HCl for 30 min at 37 °C and BrdU sites exposed by 0.01% trypsin (Sigma) for 15 min at 37 °C. Nonspecific staining was blocked with 1% bovine serum albumin (BSA). The sections were then incubated with anti-BrdU primary antibody (G3G4, Developmental Studies Hybridoma Bank, University of Iowa) overnight at 4 °C, which was followed by 2 × 5′ washes and incubation with goat anti-mouse IgG specific coupled to horseradish perioxidase (1:500, Vector) for 2 h at room temperature. The immunolabeling was then visualized using diaminobenzidine (DAB) and counterstained with hematoxylin. Total nuclei and $BrdU^+$ nuclei were counted in every second section under microscope, and the proportion of $BrdU^+$ cells to total cells in each DRG was calculated and expressed as a percentage ("proliferation index", PI). $BrdU^+$ and total cell counts were performed in 10 C2 DRG and C5 DRG on both sides of five embryos for each treatment.

2.4. Measurement of DRG volumes

Every fourth sections of the serially cut DRG were photographed using a digital camera (Leica DC100). The images were then transferred to Leica Image Database (Leica, Germany) and the areas of the DRG were measured