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雌、雄激素受体在文昌鱼神经系统和哈氏窝的免疫识别*

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摘 要 用雌、雄激素受体的多克隆抗体对不同发育时期的文昌鱼神经系统和哈氏窝进行免疫识别。结果显示, 雌、雄激素受体免疫阳性识别发生在文昌鱼端脑和中脑的中部与后部, 以及神经管的背面和中央, 后脑则为免疫阴性反应。两种激素受体免疫阳性物多数分布在神经细胞核内及其神经纤维, 少数在胞质。雌、雄激素受体免疫阳性识别还出现在文昌鱼哈氏窝基部的上皮细胞核内或胞质中。雌、雄激素受体在文昌鱼神经系统和哈氏窝的免疫识别部位与脊椎动物相类似。研究表明, 性类固醇激素像在脊椎动物中那样, 可对文昌鱼脑和哈氏窝进行反馈调节。

关键词 文昌鱼 雌、雄激素受体 免疫细胞化学 神经系统 哈氏窝

脊椎动物生殖内分泌学研究揭示, 雌、雄激素对脑和垂体的反馈调节必须与脑内相应受体结合才能进行 (Barley *et al*., 1975; Yuan *et al*., 1995)。研究证实, 脊椎动物不同进化阶段, 哺乳类 (Krey *et al*., 1982; Handa *et al*., 1987)、爬行类 (Halpern *et al*., 1982) 和硬骨鱼 (Trudeau *et al*., 1993) 的脑内都存在雌激素或雄激素受体, 在鱼类脑垂体 (Kim *et al*., 1979; Schreibman *et al*., 1982) 中也发现有这两种受体。然而, 对于进化上界于无脊椎动物与脊椎动物之间过渡类型的头索类文昌鱼, 其脑内和垂体中是否存在这两种受体仍不为所知。用放免测定 (Zhang *et al*., 1985; 方永强等, 1993) 已证实了文昌鱼性腺能够合成和分泌性类固醇激素 (雄激素、雌激素和孕激素), 但文昌鱼性类固醇激素是否像在脊椎动物中那样, 对脑和哈氏窝 (与鱼类脑垂体同源) 具有反馈调节的作用, 这在国内外均未见研究。本文用雌、雄激素受体的多克隆抗体对文昌鱼神经系统和哈氏窝进行免疫细胞化学定位, 为进一步阐明文昌鱼生殖内分泌调控轴 (脑-哈氏窝-性腺轴) 各组成部分之间的相互调节提供形态学依据。

1 材料和方法

1.1 实验动物

文昌鱼 (*Branchiostoma belcheri*) 取自厦门同安琼头海区。本实验共用文昌鱼 24 尾, 体长 45.3～52.6 mm, 雌、雄各 12 尾。按文昌鱼性腺发育周期 (方永强等, 1990) 划分, 它们分别属于小生长期 (Ⅰ～Ⅱ期)、大生长期 (Ⅲ～Ⅳ期) 和成熟期 (Ⅴ期)。

1.2 样品制备

文昌鱼经低温麻醉处理后, 每尾动物分为三部分, 头部 (从吻端至第一对性腺之前)、中部 (从第一对性腺至肛门) 和尾部。样品在新配制的不含醋酸 Bouins 液中固定 8～12 h, paraplast 包埋, 连续纵切或横切, 切片厚 6 μm, 在光镜下鉴别和选择含有脑、神经管和哈氏窝的片子, 用于免疫细胞化学反应。

1.3 免疫细胞化学反应程序

参照方永强等 (1999b) 方法, 进行 SABC (strept avidin biotin complex) 免疫细胞化学反应。兔抗雌激素或雄激素受体多克隆抗体 (美国 Santa Cruz 公司产品, 购自北京中山生物技术有限公司, 这两种抗体经该公司鉴定, 各自不与其它类固醇激素受体发生交叉反应) 的稀释度为 1∶100, 生物素标记的羊抗兔抗体 (武汉博士德公司产品) 的稀释

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度为 1∶100。对照实验片用正常兔血清替代第一抗体进行孵育，结果为阴性反应。

2 结　果

2.1　雌、雄激素受体在神经系统的分布

文昌鱼神经系统由脑和神经管组成，脑可分为端脑、中脑和后脑三部分（方永强等，1999a）。从雌、雄文昌鱼头部纵切面上，可见在端脑、中脑的中部（与哈氏窝相对，相当于鱼类下丘脑）和后部的背面，有椭圆形和不规则形神经细胞对这两种受体的多克隆抗体发生免疫阳性反应，阳性物多数定位在核内，少数在胞质，显棕褐色（图版 Ⅰ：1，2，3），后脑则显免疫阴性反应。在头部横切面上，雌、雄激素受体在脑泡神经细胞的免疫染色反应及定位的结果与纵切面相类似，也是定位在核和胞质（图版 Ⅰ：4）。在神经管横切面上，神经管背面和中央的神经细胞对雌激素和雄激素受体的多克隆抗体显免疫阳性反应，阳性物定位在神经细胞核、胞质及其神经纤维，棕褐色（图版 Ⅰ：5，6）。

2.2　雌、雄激素受体在哈氏窝的免疫识别

文昌鱼哈氏窝由三种不同类型的细胞（方永强等，1989）所组成。一种为棱形或不规则形上皮细胞，位于哈氏窝的顶部；哈氏窝中部的另一种细胞为多边形或不规则形；在哈氏窝底部，靠近口腔处为带纤毛的粘液细胞。免疫反应结果显示，雌激素和雄激素受体免疫识别仅发生在哈氏窝顶部的上皮细胞（为文昌鱼原始的促性腺激素分泌细胞）中，阳性物定位在胞质和核内（图版 Ⅰ：7，8），其余两种细胞则为阴性反应。

3 讨　论

虽然 Pfaff 等（1978）对类固醇激素在各纲脊椎动物（从鱼类至哺乳类）下丘脑的结合部位及其投射路线作过评述，但对于类固醇激素受体在脑内神经细胞的精确定位还很少了解。后来，一些学者陆续在大鼠、蛇和鱼类中证实了类固醇激素受体分布在脑的不同核群。例如，Krey 等（1982）报道雄激素受体分布在大鼠的下丘脑、视前区和杏仁核的神经细胞核或胞质。Handa 等（1987）用放射自显影发现雄激素受体分布在大鼠下丘脑下部和外周神经系统的神经细胞胞质中。Halpern 等（1982）用氚标记雌二醇和睾酮进行定位研究，发现这两种激素受体分布在蛇的端脑腹杏仁核、内侧核球、旧纹状体和视前区等。Trudeau 等（1993）研究发现，标记的睾酮和雌二醇受体分布在斑剑尾鱼和金鱼的视前区和下丘脑神经细胞的核和胞质中。本文用雌、雄激素受体的多克隆抗体进行免疫识别，首次发现文昌鱼端脑、中脑的中部（相当于鱼类下丘脑）和后部以及脊髓都有这两种受体分布，且受体阳性物多数定位在神经细胞的核，少数在胞质中。这就表明类固醇激素受体在文昌鱼脑和脊髓的定位与脊椎动物十分类似。

本文还首先发现雌、雄激素受体免疫识别发生在文昌鱼哈氏窝顶部不规则形或棱形上皮细胞中的核和胞质中。此结果与 Schreibman 等（1982）和 Kim 等（1979）分别用荧光-类固醇激素偶联法（fluorescent-steroid hormone conjugates）和放射自显影法发现雌激素和睾酮受体定位在斑剑尾鱼脑垂体促性腺激素分泌细胞的核和胞质以及近 80 %免疫活性 LH 细胞显示放射活性聚集在核中相一致。

性类固醇激素是脊椎动物下丘脑-脑垂体功能发育和成熟的重要调节者，在哺乳类、鸟类和鱼类已得到证实（Kalra *et al*.，1983；Fink *et al*.，1988）。我们先前研究已证实，文昌鱼具有原始的生殖内分泌调控轴（方永强，1998），但不知道该调控轴功能的成熟与维持是否如脊椎动物那样，依赖于类固醇激素的激发和调控。现在发现文昌鱼脑和哈氏窝存在雌、雄激素受体，这就为类固醇激素可能对文昌鱼脑和哈氏窝具有反馈调控以及类固醇激素可能激发和调控文昌鱼生殖调控轴功能的发育成熟与维持提供形态学依据。因而，本文研究结果从深层次上进一步揭示头索动物文昌鱼生殖内分泌调控轴各部分之间相互关系与脊椎动物的相似性，在理论上有重要的意义。

另一方面，脊椎动物的生殖内分泌研究揭示，类固醇激素还具有调节性腺功能和生殖行为的作用。在大鼠（Krey *et al*.，1982；Handa *et al*.，1987）、蛇（Halpern *et al*.，1982）、斑剑尾鱼（Kim *et al*.，1979；Schreibman *et al*.，1982）和金鱼（Trudeau *et al*.，1993）中，雌激素和睾酮受体分布在这些动物的视前区、下丘脑和脑垂体，而这些部位是参与调节动物分泌促性腺激素的。现已知文昌鱼具有原始的生殖内分泌调控轴：脑-哈氏窝-性腺轴，本文研究又发现，雌、雄激素受体定位在文昌鱼的中脑中部（相当于鱼类的下丘脑）和哈氏窝（原始的脑垂体），因此我们认为文昌鱼类固醇激素可能也具有类似的机制调节其性腺功能。同时，文昌鱼脑和哈氏窝存在雄激素受体还能

够较完满地解释我们以前的发现，外源性类固醇激素 17α-甲基睾酮能够刺激文昌鱼精子发生和精子形成（方永强等，1991）。其机制既有直接激发精巢精子发生作用，又可能是经脑内类固醇激素受体，通过正反馈作用实现的。

虽然本文证实了文昌鱼性类固醇激素通过脑和哈氏窝受体的介导而行使反馈调节，但其内在机制及环节是否与脊椎动物相似，仍不十分清楚。例如，睾酮必须在鱼类脑内的特定部位，通过芳香化酶（aromatase）转换为雌二醇而起作用（Pasmanik *et al*.，1985），而文昌鱼脑内是否存在这种酶，还有待证实。又如，雌激素和雄激素受体定位在文昌鱼端脑中部和后部，有何生理意义，也尚未明了。这些重要且有趣的问题有待深入研究。

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外　文　摘　要 (Abstract)

IMMUNORECOGNITION OF ESTROGEN AND ANDROGEN RECEPTORS IN THE NERVOUS SYSTEM AND HATSCHEK'S PIT OF AMPHIOXOUS (BRANCHIOSTOMA BELCHERI) *

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Previous studies discovered that Amphioxus (*Branchiostoma bekheri*) had primitive reproductive endocrine regulation axis (brain-Hatschek's pit-gonads) which synthesizes and secretes sex steriod hormones (estrogen, androgen and progestergone). However, the feedback regulation of sex steroid hormones on brain and Hatschek's pit (homologous with the pituitary gland) of Amphioxus had not been reported so far. Thus, we used polyclonal antibodies against estrogen and androgen receptors to investigate the immuno-recognition of estrogen and androgen receptors in nervous system and Hatschek's pit of Amphioxus at different development stages.

Amphioxus were collected from the Tong'an-Qiongtou sea area near Xiamen. Each fish was divided into three segments, head (from tentaculum peristomial to the first pair of gonads), medial (from the first pair of gonads to the amus) and tail. Samples were fixed in Bouin's solution without acetic acid for 8～12 h, then embedded in paraplast. Sections with the structure of brain and Hatschek's pit were identified under a light microscope and selected for immunostaining with the SABC technique.

The results showed that the immuno-recognition of estrogen and androgen receptors existed in the telencephalon and the middle and posterior part of midbrain. Immunopositive substances mostly located in the nucleus of nerve cells, with a few in the cytoplasm. The dorsal side and middle region of nerve tube also showed immunopositive reaction, and the immunopositive substances of estrogen and androgen receptors distributed in the nucleus, cytoplasm and nerve fibers of nerve cells. But immunonegative reaction was showed in the hindbrain. On the other hand, immuno-recognition of estrogen and androgen receptors existed in the nucleus or the cytoplasm of epithelial cells (primitive gonadotrophic cells in Amphioxus) in the base part of Hatschek's pit at different stages of development. The other two cells in Hatschek's pit showed immunonegative reaction.

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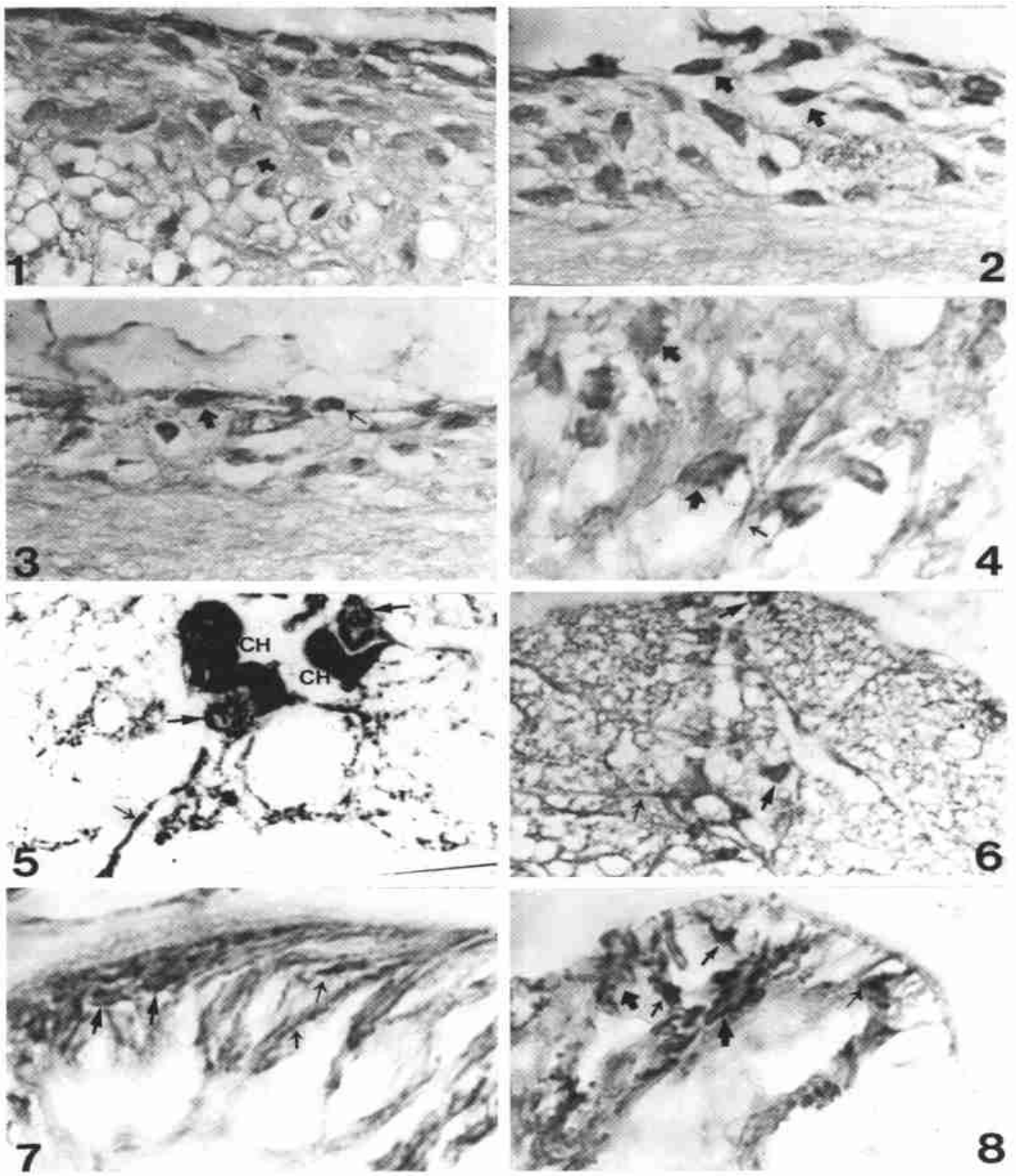
The results demonstrate for the first time that estrogen and androgen receptors exist in cephalochordata Amphioxus, and the immunorecognitive regions of the estrogen and androgen receptors in the brain and Hatschek's pit are similar to those of vertebrates. It suggests that sex steroid hormones exert feedback regulation on brain and Hatschek's pit, and stimulate the development and maturity of the reproductive endocrine regulation axis function in Amphioxus. These facts further demonstrate a system of reproductive endocrine regulation in Amphioxus similar to, or with close relationship with that in vertebrate. On the other hand, we suggest that the regulatory role of sex steroid hormones on gonads function and reproduction behavior may be direct or indirect through sex steroid hormones receptors in brain.

Key words Amphioxus (*Branchiostoma belcheri*), Estrogen and androgen receptor, Immuocytochemistry, Nervous system, Hatschek's pit

图版说明 (Explanation of Plate)

图 版 I (Plate I)

1. 卵巢发育 III~ IV期的文昌鱼，头部纵切面，雌激素受体多克隆抗体免疫阳性物分布在端脑后部背面大的不规则形神经细胞核内（粗箭头），少数分布在胞质（细箭头） [Amphioxus at ovary III~ IV stage, longitudinal section through head region, immunopositive substance of polyclonal antibodies of estrogen receptor were recognized in the nucleus (thick arrow) and a few in the cytoplasm (thin arrow) of large irregular nerve cells at the dorsal side of posterior part of telencephalon] ×400
2. 精巢发育 IV期的文昌鱼，头部纵切面，雄激素受体免疫阳性物分布在中脑中部背面的神经细胞核内（箭头） [Amphioxus at testis IV stage, longitudinal section through head region, immunopositive substance of androgen receptor distributed in the nucleus (arrow) of nerve cells at the dorsal side of middle part of midbrain] ×400
3. 精巢发育 II期的文昌鱼，头部纵切面，雄激素受体免疫阳性物分布在中脑后部背面的中等（粗箭头）和小型（细箭头）神经细胞核内 [Amphioxus at testis III stage, longitudinal section through head region, immunopositive substance of androgen receptor distributed in the nucleus of middle (thick arrow) and small (thin arrow) nerve cells at the dorsal side of posterior part of midbrain] ×400
4. 精巢发育 II期的文昌鱼，头部横切面，雌激素受体免疫阳性物分布在脑泡背面和中央的神经细胞核或胞质（粗箭头）及其神经纤维（细箭头） [Amphioxus at testis III stage, cross section through head region, immunopositive substance of estrogen receptor distributed in the nucleus or cytoplasm (thick arrow) and nerve fibers (thin arrow) of nerve cells at the dorsal side and middle region of brain vesicle] ×500
5. 卵巢发育 II期的文昌鱼，神经管横切面，雌激素受体免疫阳性物分布在神经管中央，靠近色素细胞的神经细胞核（粗箭头）及其神经纤维（细箭头） chr: 色素细胞 (Chromocyte) [Amphioxus at ovary II stage, cross section through nerve tube, immunpositive substance of estrogen receptor distributed in the nucleus (thick arrow) and nerve fibers (thin arrow) of nerve cells near chromocyte at the middle region of nerve tube] ×400
6. 卵巢发育 IV期的文昌鱼，神经管横切面，雄激素受体免疫阳性物定位在神经管背面和中央的小型的神经细胞核或胞质（粗箭头）及其神经纤维（细箭头） [Amphioxus at ovary IV stage, cross section through nerve tube, immunpositive substance of androgen receptor located in the nucleus or cytoplasm (thick arrow) and nerve fibers (thin arrow) of small nerve cells at the dorsal side and middle region of nerve tube] ×400
7. 卵巢发育 V期的文昌鱼，通过哈氏窝横切面，雌激素受体分布在哈氏窝顶部不规则形上皮细胞（粗箭头）和棱形上皮细胞（细箭头）的胞质中 [Amphioxus at ovary V stage, cross through Hatschek's pit, estrogen receptors distributed in the cytoplasm of irregular (thick arrow) and long (thin arrow) epithelial cells at the top region of Hatschek's pit] ×500
8. 精巢发育 II期的文昌鱼，通过哈氏窝横切面，雄激素受体分布在哈氏窝上皮细胞的胞质（粗箭头）和核内（细箭头） [Amphioxus at testis II stage, cross through Hatschek's pit, androgen receptors distributed in the cytoplasm (thick arrow) and nucleus (thin arrow) of epithelial cells of Hatschek's pit] ×500



图版说明见文后 (Explanation at the end of the text)