Published in final edited form as:

Ann N Y Acad Sci. 2005 April ; 1040: 332–336. doi:10.1196/annals.1327.054.

Neurodistribution of Androgen Receptor Immunoreactivity in the Male Frog, *Rana esculenta*

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Abstract

Sexual behavior in vertebrates depends on the cyclic release of steroids and their binding to the brain receptors. Previously, we demonstrated the presence of specific binding of ³H-testosterone and staining with PG-21 in the brain of the adult male frog, *Rana esculenta*. Here, we report our further receptor characterization using an anti-androgen receptor antiserum, PG-21, and the androgen site of action in frog brain. Nuclei, which contained cells labeled for the androgen receptor (AR), were mainly identified in the olfactory bulbs, preoptic-septal region, infundibulum, amygdala, thalamus, tectum, torus semicircularis, and medulla. The neuroanatomical AR staining appears similar to that in other lower vertebrates.

Keywords

androgen receptor; amphibian; brain; PG-21

INTRODUCTION

Throughout vertebrate classes, androgens exert important functions in male reproduction.¹ Autoradiography has been used successfully to describe androgen-concentrating neurons in mammals, birds, reptiles, amphibians, and fish.² Thus, implants of testosterone in the rostral hypothalamus of castrated *Rana pipiens* restore male sexual behavior.³ The mechanism of action of androgens in the brain is related to aromatase activity,⁴ and binding with estrogen and androgen receptors is believed to be an initial event in the mechanism of central androgenic mediated action.⁵ In the present study, the neuroanatomical distribution of androgen receptor (AR) immunoreactivity (ir) has been investigated in the brain of the adult male frog, *Rana esculenta*, using an anti-AR antiserum.

MATERIALS AND METHODS

Adult male frogs, *Rana esculenta*, collected in the breeding season were submitted to transcardial perfusion with saline and the brains were removed immediately following decapitation. For immunohistochemical procedures, the brains were dissected, fixed, cryoprotected, and stored in liquid nitrogen until sectioned. For biochemical procedures, the hypothalamic region was sectioned under a stereomicroscope using the following margins: anterior, septo-mesencephalic tract; posterior, where the third nerve enters the brain; dorsal,

the level of the anterior commissure. Hypothalamic and extrahypothalamic areas were pooled into two different groups. Preparation of cytosolic and nuclear extracts of hypothalamic and extrahypothalamic areas was performed according to standard procedures reported in ref. ⁵. The anti-AR specificity of the PG-21 serum was tested, using immunohistochemical and biochemical studies, by omission of the primary antiserum, with a biotinylated antirabbit IgG, by competition studies^{6,7} and by Western blotting.⁸

RESULTS

Immunostaining only appeared in cell nuclei, whereas no detectable reaction product could be observed in the cytoplasm. FIGURE 1 shows that AR-ir is widely distributed throughout the brain, namely, in the olfactory bulbs (OB), striatum (ST), septum (SP), amygdala (AMY), thalamus (TH), preoptic area (POA), tectum (TEC), torus semicircularis (TOR), infundibulum (INF), pituitary (PIT), interpeduncular nucleus (IPN), cerebellum (CB), and motor nuclei of the medulla (MED). Highest density was observed in the hypothalamus. FIGURE 2 demonstrates immunoreactivity in the cytosolic and nuclear extracts of the hypothalamic and extrahypothalamic areas of the male frog, *Rana esculenta*, with PG-21 and the antirabbit IgG. PG-21 cross-reacted only in the nuclear extract of both brain areas, with a band corresponding to a molecular weight of about 110 kDa.

DISCUSSION

Our results show the distribution of AR-ir in the brain of a lower vertebrate: the anuran, *Rana esculenta*. The major areas of AR-ir in the male brain of the breeding period are the OB, SP, ST, AMY, POA, TEC, TOR, INF, and MED. A similar pattern is reported in the brain of other lower vertebrates: the urodele, *Taricha granulosa*;⁹ and the reptile, *Sceloporus undulatus*.⁶ Furthermore, as in urodela, ARs in anura occur in more brain regions than in mammals and fish.^{10–13} No autoradiographic evidence has been provided for the androgen site in TH, PIT, IPN, and CB of *Rana esculenta*.¹⁴ AR in vertebrates shows a large variability in molecular weight, but Western analysis in *Rana esculenta* reveals one immunoreactive band, with an apparent molecular weight of about 110 kDa. Our data reinforce our previous biochemical studies of androgen-binding molecules in the nuclear extract of frog brain with the characteristics of a true receptor¹⁵ and with the majority of the ³H-T binding activity attributable to the hypothalamic area. Further research may help to define the role of the AR in different brain areas and throughout the reproductive cycle.

REFERENCES

1. Brown, TR. Encyclopedia of Reproduction. Vol. Volume 4. San Diego: Academic Press; 1999. Steroid hormones: overview; p. 634–644.
2. Gahr M. Localization of androgen receptors and estrogen receptors in the same cells of the songbird brain. Proc. Natl. Acad. Sci. USA 1990;87:9445–9448. [PubMed: 2251286]
3. Schmidt RS. Preoptic activation of frog mating behavior. Behaviour 1968;30:239–257.
4. Lephart ED. A review of brain aromatase cytochrome P450. Brain Res. Rev 1996;22:1–26. [PubMed: 8871783]
5. Guerriero G, Roselli CE, Paolucci M, et al. Estrogen receptor and aromatase activity in the hypothalamus of the female frog, *Rana esculenta*: fluctuations through the reproductive cycle. Brain Res 2000;880:92–101. [PubMed: 11032993]
6. Moga MM, Geib BM, Zhou D, Prins GS. Androgen receptor-immunoreactivity in the forebrain of the Eastern Fence lizard (*Sceloporus undulatus*). Brain Res 2000;879:174–182. [PubMed: 11011019]
7. Prins GS, Birch L, Greene GL. Androgen receptor localization in different cell types of the adult rat prostate. Endocrinology 1991;129:3187–3199. [PubMed: 1954898]

8. Guerriero G, Ciarcia G. Progesterone receptor: some viewpoints on hypothalamic seasonal fluctuations in a lower vertebrate. *Brain Res. Rev* 2001;37:172–177. [PubMed: 11744085]
9. Davis GA, Moore F. Neuroanatomical distribution of androgen and estrogen receptor-immunoreactive cells in the brain of the male roughskin newt. *J. Comp. Neurol* 1996;372:294–308. [PubMed: 8863132]
10. Callard GV. Androgen and estrogen actions in the vertebrate brain. *Am. Zool* 1983;23:607–620.
11. Roselli CE, Handa RJ, Resko JA. Quantitative distribution of nuclear androgen receptors in microdissected areas of the rat brain. *Neuroendocrinology* 1989;49:449–453. [PubMed: 2725839]
12. Clancy AN, Whitman C, Michael RP, Albers HE. Distribution of androgen receptor-like immunoreactivity in the brains of intact and castrated male hamsters. *Brain Res. Bull* 1994;33:325–332. [PubMed: 8293317]
13. Gelinas D, Callard GV. Immunolocalization of aromatase and androgen receptor-positive neurons in the goldfish brain. *Gen. Comp. Endocrinol* 1997;106:155–168. [PubMed: 9169111]
14. di Meglio M, Morrell JI, Pfaff DW. Localization of steroid-concentrating cells in the central nervous system of the frog *Rana esculenta*. *Gen. Comp. Endocrinol* 1987;67:149–154. [PubMed: 3497838]
15. Guerriero, G.; Prins, GS.; Birch, L.; Ciarcia, G. Trends in Comparative Endocrinology. Japan: AOSCE; 2004. Androgen receptor: a seasonal update in the brain of the male frog, *Rana esculenta*; p. 289-291.

FIGURE 1.

Mapping of AR-ir in the brain of the male frog, *Rana esculenta*, captured in the breeding season, using the anti-AR antiserum, PG-21. Terms: OB, olfactory bulbs; ST, striatum; SP, septum; AMY, amygdala; TH, thalamus; POA, preoptic area; TEC, tectum; TOR, torus semicircularis; INF, infundibulum; PIT, pituitary; IPN, interpeduncular nucleus; CB, cerebellum; MED, medulla.

FIGURE 2.

Western blotting of the hypothalamus and extrahypothalamus of the male frog, *Rana esculenta*, captured in the breeding period, incubated with PG-21 and with antirabbit IgG as control. The experiment was performed at least three times with cytosolic (cyt) and nuclear extracts (n.e.) from different animals, which gave similar results.