ESTROGEN RECEPTOR-α IMMUNOREACTIVE NEURONS IN THE BRAINSTEM AND SPINAL CORD OF THE FEMALE RHESUS MONKEY: SPECIES-SPECIFIC CHARACTERISTICS

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Abstract—The distribution pattern of estrogen receptors in the rodent CNS has been reported extensively, but mapping of estrogen receptors in primates is incomplete. In this study we describe the distribution of estrogen receptor alpha immunoreactive (ER-α IR) neurons in the brainstem and spinal cord of the rhesus monkey.

In the midbrain, ER-α IR neurons were located in the periaqueductal gray, especially the caudal ventrolateral part, the adjacent tegmentum, peripeduncular nucleus, and pretectal nucleus. A few ER-α IR neurons were found in the lateral parabrachial nucleus, lateral pontine tegmentum, and pontine gray medial to the locus coeruleus. At caudal medullary levels, ER-α IR neurons were present in the commissural nucleus of the solitary complex and the caudal spinal trigeminal nucleus. The remaining regions of the brainstem were devoid of ER-α IR neurons. Spinal ER-α IR neurons were found in laminae I–V, and area X, and were most numerous in lower lumbar and sacral segments. The lateral collateral pathway and dorsal commissural nuclei of the sacral cord and the thoracic intermediolateral cell column also contained ER-α IR neurons. Estrogen treatment did not result in any differences in the distribution pattern of ER-α IR neurons.

The results indicate that ER-α IR neurons in the primate brainstem and spinal cord are concentrated mainly in regions involved in sensory and autonomic processing. Compared with rodent species, the regional distribution of ER-α IR neurons is less widespread, and ER-α IR neurons in regions such as the spinal dorsal horn and caudal spinal trigeminal nucleus appear to be less abundant. These distinctions suggest a modest role of ER-α in estrogen-mediated actions on primate brainstem and spinal systems. These differences may contribute to variations in behavioral effects of estrogen between primate and rodent species. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

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Estrogen modulates neural activity related to a wide variety of physiological events, such as reproductive behaviors (Ogawa et al., 1998), lactation, maternal behaviors, vocalization (Geyer and Barfield, 1978), aggressive behavior (Ogawa et al., 1996), autonomic functions (Saleh et al., 2000), analgesia (Bodnar et al., 2002), levels of motor activity (Ogawa et al., 2003), food intake (Geary et al., 2001), memory and cognitive functions (Fink and Sumner, 1996; Sherwin, 2000). The above functions are under control of estrogen sensitive hypothalamic and forebrain regions, though modulation takes place via brainstem and/or spinal cell groups. Examples are food intake being modulated by the nucleus of the solitary tract (NTS), cognitive functions by the dorsal raphe nucleus (DRN), vocalization and reproductive behavior by the periaqueductal gray (PAG), and nociception by the spinal dorsal horn and the ventromedial medulla (see VanderHorst et al., 2005).

Most of the data on the effects of estrogen on physiological functions have been obtained in rodents. The few reports involving primates have shown that reproductive behavior and nociception are not modulated by estrogen as much as seen in rodents (Chambers and Phoenix, 1987; Negus et al., 2004), and the same may be true for other actions that are modulated by estrogen. The neuronal basis underlying this differential effect of estrogen on physiological functions is not well understood, but may include differences in the ability of brainstem and spinal neurons to respond to estrogen.

Estrogen-induced behavioral effects might be mediated by populations of neurons that express estrogen receptors. A number of estrogen receptors have been identified, which may mediate their action via genomic and/or non-genomic mechanisms (for review see Vasudevan and Pfaff, 2008). Estrogen receptors-alpha and -beta (ER-α and ER-β) are most abundant in the nucleus though they can also be found at extra-nuclear sites. In addition, recently a possible role in estrogen-mediated actions has been described for the G protein–coupled receptor 30 (GPR30), which is located in plasma membrane, Golgi apparatus and endoplasmic reticulum (Revankar et al., 2005; Thomas et al., 2005; Noel et al., submitted for publication; for review see Vasudevan and Pfaff, 2008).

The distribution of neurons expressing the nuclear estrogen receptors (ER-α and ER-β) in the rodent CNS has

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Abbreviations: ABC, avidin–biotin-complex-peroxidase; DRN, dorsal raphe nucleus; ER-α, estrogen receptor-alpha; ER-β, estrogen receptor-beta; ER-α IR, estrogen receptor-alpha immunoreactive; GPR30, G protein–coupled receptor 30; NTS, nucleus of the solitary tract; PAG, periaqueductal gray; PBS, phosphate-buffered saline.

been mapped extensively by immunohistochemical or *in situ* hybridization methods (Simerly et al., 1990; Turcotte and Blaustein, 1993; Shughrue et al., 1997; Boers et al., 1999; Merchenthaler et al., 2004; VanderHorst et al., 2005). In the rodent brainstem and spinal cord, large numbers of neurons express ER-α, whereas few neurons express the high affinity isoform of ER-β (Merchenthaler et al., 2004; VanderHorst et al., 2005). The distribution of GPR30 immunoreactive neurons has been recently described in the rat CNS, including midbrain and medulla oblongata (Brailoiu et al., 2007).

The above rodent studies showed some species differences in the distribution patterns of estrogen receptor-alpha immunoreactive (ER-α IR) neurons, especially at the level of the brainstem. It is likely that there are substantial species differences between the primate and rodent brainstem. Only a few reports deal with the distribution of estrogen concentrating neurons throughout the CNS in primates (using autoradiographic techniques: Keefer and Stumpf, 1975, Pfaff et al., 1976). More recent studies in the primate, using *in situ* hybridization or immunohistochemical techniques, focus on hypothalamic and forebrain areas (Herbison et al., 1995; Register et al., 1998; Blurton-Jones et al., 1999), the PAG (ER-α; VanderHorst et al., 2002b), DRN (ER-β: Gundlah et al., 2000, 2001) and locus coeruleus (Pau et al., 2000). The distribution pattern of ER-IR neurons in the rest of the primate brainstem and the spinal cord has not been reported in detail. Such information is essential if we are to understand how and to what extent estrogen, acting via estrogen receptors, is involved in the modulation of brainstem and spinal circuitries in primates.

Here we report the distribution of ER-α IR neurons in the midbrain, brainstem and spinal cord of the adult female rhesus monkey. For inter-species comparison, methodology and tissue analysis were similar to a recent study on the distribution of ER-α IR neurons in the mouse (VanderHorst et al., 2005).

EXPERIMENTAL PROCEDURES

Animals and surgical procedures

Seven adult female rhesus monkeys (*M. mulatta*; weight: 4.7–7.5 kg; age 8–26 years, average age 15 years; cases M11, M12, and M14 to M18) were used (see Table 1 for details). The protocol for this study was reviewed and approved by the Animal Care and Use Committee, University of Wisconsin, Madison, and all experiments were performed under the guidelines established by the NIH and USDA (NIH Guide for the Care and Use of Laboratory Animals; NIH Publications No. 80-23). All animals have been included in other studies (VanderHorst et al., 2000a,b, 2001b, 2002a,b, 2004) to minimize the number of animals.

To eliminate variations in immunostaining related to variations in menstrual cycle, ovariectomies had been performed in all cases, except for one monkey (M18). Given her age of 26 years as well as based on a menstrual record, the endocrine status of this monkey was postmenopausal. Ovariectomies were performed well in advance of the euthanization of the animals (6 weeks: M11 and M12; 3 months: M16 and M17; more than a year: M14 and M15). Three animals received daily s.c. injections of estradiol benzoate (M14, M16, M17; 40 μg; Sigma, St. Louis, MO, USA) for 14 days prior to euthanasia. This dose is sufficient to facilitate mating behavior in female rhesus monkeys (Chambers and Phoenix, 1987). The three estrogen treated cases were included to examine whether 14 days of estradiol benzoate treatment would affect the distribution of ERα-IR staining.

At the time of euthanasia the animals were anesthetized with ketamine (10–15 mg/kg; i.m.) and pentobarbital (25–30 mg/kg; i.v.), and transcardially perfused with 2 liters of phosphate-buffered saline (PBS; pH 7.4; 0.1 M; room temperature) followed by 2–3 liters of fixative. The fixative contained 0.5% glutaraldehyde (EMS, Fort Washington, PA, USA) and 4% paraformaldehyde (EMS) in 0.1 M phosphate buffer (pH 7.4; cases M11 and M12), 1.5% glutaraldehyde and 1.5% paraformaldehyde (cases M14 to M17), or 4% paraformaldehyde (M18). The high glutaraldehyde fixation in M14 to M17 was necessary for tracing experiments that were conducted in these animals as part of other studies (VanderHorst et al., 2000a, 2002b).

The CNS was removed, post-fixed for 2–4 h, and stored in PBS (M11 to M17) or 25% sucrose in PBS (M18; both at 4 °C). The midbrain, brainstem and spinal segments C1, C3, C7, T2, T12, L2, L4, L5, L6, L7, S1, S2, and S3 were blocked and each block was cut into 10 series of 50 μm sections on a vibratome (M11 to M17) or a freezing microtome (M18).

ER-α immunohistochemistry

For visualization of ER-α IR neurons, two primary antibodies were used. The H222 antibody (gift by Abbott Laboratories, Chicago, IL, USA; 1.0 μg/ml, 1:1000) is directed against the ligand-binding domain of ER-α. The specificity of the H222 antibody has been documented previously (see King and Greene, 1984). Using the H222 antibody, the distribution of ER-mRNA and ER-IR was similar in the rhesus monkey hypothalamus (Bethea et al., 1996). Theoretically, the H222 antibody might also detect ER-β. Therefore, an additional experiment was performed using the 1D5 antibody (monoclonal mouse anti-human estrogen receptor; DAKO, Glostrup, Denmark; 230 μg/ml at a dilution of 1:400), which is directed against the N-terminus of ER-α, a region that does not overlap with ER-β. In rat (Greco et al., 1998) and monkey (VanderHorst et al., 2002b) the distribution of ER-IR neurons in the CNS is similar with these two antibodies. Moreover, ER-beta

Table 1. Overview of experimental parameters

Case	Age (yrs)	OVX (wks)	Estradiol benzoate	Fixative	Primary antibody
M11	18	6	No	4% PF, 0.5% GA	H222
M12	12	6	No	4% PF, 0.5% GA	H222
M14	8	>52	Yes	1.5% PF, 1.5% GA	H222
M15	10	>52	No	1.5% PF, 1.5% GA	H222
M16	11	12	Yes	1.5% PF, 1.5% GA	H222
M17	18	12	Yes	1.5% PF, 1.5% GA	H222
M18	26	None	No	4% PF	1D5

OVX, ovariectomy, interval between ovariectomy and perfusion; GA, glutaraldehyde; PF, paraformaldehyde.