Developmental expression of the estrogen receptor-related receptor γ in the nervous system during mouse embryogenesis

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Received 4 May 2000; received in revised form 15 June 2000; accepted 17 July 2000

Abstract

The ERR’s (estrogen receptor-related receptors) are constitutive activators of the classical estrogen response element. In this report, we demonstrate that ERRγ is highly expressed in the nervous system of the developing mouse embryo and that the adult pattern of expression of ERRγ is, with few exceptions, established during embryogenesis. Transcripts are preferentially detected in already differentiating areas of the nervous system.

Keywords: Orphan nuclear receptor; Estrogen receptor-related receptor; Expression pattern; Mouse embryogenesis; In situ hybridization; Nervous system

1. Results

ERRs are orphan nuclear receptors sharing high sequence homology with estrogen receptors, but have no known ligands. ERRγ cDNAs were isolated as a candidate disease gene for Usher syndrome type IIa, in a project to isolate large cDNAs, by bioinformatics, and in a two-hybrid screening (Eudy et al., 1998; Nagase et al., 1998; Chen et al., 1999; Hong et al., 1999). In the adult mouse, ERRγ is expressed in heart, brain, muscle and kidney (Hong et al., 1999; Süsens et al., 2000). In situ hybridization on adult mouse brain sections shows strong and differential expression of ERRγ transcripts in the isocortex, the olfactory system, cranial nerve nuclei, major parts of the coordination centers, all nuclei involved in acoustic reflexes, and in sensory ganglia (Lorke et al., 2000). Northern blot analysis reveals ERRγ transcripts of approximately 5.7 kb at embryonal day 11 (E11), E15, and E17 (Hong et al., 1999; Süsens et al., 2000). In the present study, we ask how the adult pattern of ERRγ expression in the brain is established during embryogenesis.

Starting at E10.5 we observe expression of ERRγ in two stripes of the neuroepithelium laying beneath the midline and decreasing in intensity in a rostral to caudal fashion (Fig. 1A). At E11.5 whole-mount hybridization reveals enhanced expression of ERRγ in the floor of the mesencephalon extending caudally throughout the met- and myelencephalon (Fig. 1B). Frozen sections of the brain at the same stage of development show that these hybridization signals arise from areas were differentiation of neurons is already in progress (Fig. 1C,D). Motoric nuclei of the hindbrain and the area were the noradrenergic/adrenergic nuclear complex reside express ERRγ (Fig. 1D). At E12.5 overall signal intensity in the brain has increased and the rhombic isthmus is strongly labeled (Fig. 1E). At E13.5, in the cerebral cortex hybridization intensity has decreased compared to E12.5 (Fig. 1E-H). The olfactory bulb expresses ERRγ (Fig. 1F-H). Prominent expression is observed in the ventricular and basal aspects of the ganglionic eminence (Fig. 1H). High levels of ERRγ transcripts are detected over the lateral thalamus. In the mid- and hindbrain region the interpeduncular nucleus, the tegmental and pontine nuclei are marked by ERRγ expression (Fig. 1G). At the same time the expression in the hindbrain nuclei has become more distinct and reveals the adult pattern of expression (data not shown). At E16.5 the frontal and the parietal cortex show weak hybridization signals (Fig. 1I,J). The caudate putamen is weakly labeled while the globus pallidus is highlighted by prominent expression (Fig. 1J). Accumulation of transcripts is observed over the lateral thalamus (Fig. 1I), the reticular, and the dorsal nucleus of the thalamus (Fig. 1J,K). In the mid- and hindbrain area signal intensity has increased. The nucleus raphe and the inferior colliculus express high levels of ERRγ (Fig. 1J,K). The cerebellar cortex shows diffuse signals, while the deep cerebellar nuclei are strongly labeled.
At E18.5 signal intensity over the parietal cortex has increased (Fig. 1K). In the hippocampal formation where expression is observed in some of the pyramidal cells and interneurons in the adult, no signal is detected. The caudate putamen, which does not exhibit hybridization signals in the adult, is still labeled (Fig. 1L). The reticular nucleus of the thalamus, the zona incerta, the supramammary, the geniculate, the red, the interpeduncular, and the principal sensory trigeminal nucleus strong accumulation of silver grains as does the nucleus Darkschewitsch (Fig. 1L–N). The inferior colliculus is more intensely labeled than the superior colliculus (Fig. 1O). In the cerebellar
cortex where in the adult strong expression is seen over stellate and basket cells of the molecular layer and Golgi-cells of the granular cell layer signal intensity has increased compared to E16.5 (Fig. 1O). The external granular cell layer does not show ERRγ expression.

In the optic cup weak signals are detected at E11.5 (data not shown). Up to E16.5 they have increased in intensity and patchy hybridization signals are detected over all sensory layers of the retina (Fig. 2A,B) with less signals over the outer nuclear layer. In the otic vesicle transcripts are already detected at E10.5 (Fig. 1A). At E16.5, strong accumulation of transcripts is observed in the cochlear and the vestibular ganglion which increases forwards E18.5 (Fig. 3).

2. Experimental procedures

2.1. In situ hybridization

In situ hybridization on embryos from natural matings between inbred CD-1 mice was performed as described (Süssens et al., 1997). Probes were as described (Lorke et al., 2000). Antisense RNA probes labeled with [α-35S] UTP (1000-1500 Ci/m mole, NEN) or Digoxigenin (Roche) were generated with T7 polymerase (Ambion) according to the manufacturers instructions. Specificity of the signals was verified by using sense probes. A non-overlapping probe yielded the same hybridization pattern. Morphological structures in embryonic mice sections were identified by reference to Rugh (1990); Kaufman (1992).

Fig. 2. Expression of ERRγ in the retina. (A) emulsion-dipped section through a retina at E16.5 hybridized to the radiolabeled ERRγ riboprobe and magnification of a retina at the same stage (B) showing patchy hybridization signals over the neural layers. slr, sensory layer of the retina; v, vitreous.

Fig. 3. Expression of ERRγ in the auditory system. (A,B) Emulsion-dipped sections through the inner ear at E16.5 (A) and E18.5 (B) are shown revealing expression of ERRγ in the cochlear and vestibular ganglion. c, cochlear ganglion; v, vestibular ganglion.

Acknowledgements

The authors would like to thank Professor H. Chica Schaller for the support of the work in her institute. Thanks also to Björn Riedel for helpful discussion and Simon Hempel for help with the figures.

References


