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Dopamine receptors in equine ovarian tissues

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Abstract

Dopamine (DA) agonist and antagonist treatments can affect ovarian reproductive events in the mare. To support our theory that DA produces these effects by acting directly on the ovary, we analyzed equine ovarian tissues for the presence of dopamine receptor-1 (D1r) and dopamine receptor-2 (D2r) mRNA by reverse transcription polymerase chain reaction (RT-PCR) and D1r and D2r proteins by Western blot and immunohistochemistry (IHC). RT-PCR was performed on RNA isolated from ovarian cortex, medulla, granulosa/theca or corpus luteum (CL) tissues and from pituitary (D2r control) and renal artery (D1r control). D1r and D2r specific primers were designed from partial DNA sequences known for the horse (D2r) or conserved sequences from other species (D1r). Western blot analyses were conducted on CL, cortex and granulosa/theca samples and IHC was performed on CL tissues using D1r or D2r specific antibodies. The incidence of positive D2r mRNA was high in CL and ovarian cortex, low in granulosa/theca, and not detectable in ovarian medulla. Dopamine D1r mRNA incidence was high (50%) only in CL tissues. D1r and D2r antibody staining was positive for each tissue type analyzed by Western blot procedures. All CL tissues prepared by IHC showed positive staining for D1r and D2r proteins. Both DA receptor proteins appeared uniformly distributed throughout the CL tissue. These results indicate that equine ovarian tissues do possess D1r and D2r, and suggests that DA can act directly on ovarian tissues through its interaction with DA receptors.
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1. Introduction

The primary controllers of gonadal function are traditionally accepted to be various endocrine/paracrine mechanisms involving the hypothalamic-pituitary-gonadal axis. However, a growing collection of evidence points to an additional mechanism controlling gonadal function involving autonomic (catecholaminergic) neuronal activity, and possibly endocrine-like effects produced from neurotransmitting chemicals secreted from non-neuronal ovarian cells.

Stephenson et al. [1] mapped the autonomic innervation of the follicular-phase ovary in 12 mammalian species. A dense adrenergic nerve supply to the theca and evidence of dopamine (DA) secreting nerve cells to the graafian follicle was documented for the cow and sheep. Bahr et al. [2] also reported that DA-containing nerve terminals exist in the thecal cell layer and around the walls of mature follicles in guinea pigs, but not in the granulosa cell layer. The discovery of gonadotropin-dependent neurotropin production by antral follicles in the ewe [3] provided evidence for the method by which dynamic control of neuronal proliferation of ovarian structures may be achieved.

Indirect evidence suggests local dopaminergic mechanisms affect the ovary. Heifers fed endophytic fescue containing dopamine agonist ergot alkaloids displayed differences in estrous cycle durations, circulating progesterone concentrations and gene expression that was ameliorated by the D2r antagonist, domperidone [4,5]. Domperidone does not cross the blood–brain barrier [6] suggesting a local effect at the ovary.

In order for DA to have direct effects at the ovarian level in the mare, DA receptors must be present. The objective of the present experiments was to obtain evidence for the existence of DA receptors in equine ovarian tissues.

2. Materials and methods

2.1. Animals/samples

Samples for the determination of dopamine receptor-1 (D1r), and dopamine receptor-2 (D2r) mRNAs were collected during the height of the equine breeding season (10 July 2000). Twenty-seven ovaries were collected from 16 mares at an equine abattoir. Mare age, prior reproductive histories, and stage of cycle were unknown. Follicle diameters were measured externally prior to processing ovarian tissues. Samples of ovarian medulla ($n = 18$), cortex ($n = 19$), granulosa/theca ($n = 27$) and luteal ($n = 25$) tissues were excised. In addition, equine pituitary and renal artery samples were collected as D2r and D1r mRNA controls, respectively. Samples were transferred to labeled cryovials and immediately frozen in liquid nitrogen. Samples were maintained in liquid nitrogen until used for RNA extraction and subsequently transferred to $-80\,^{\circ}C$ for longterm storage.

Stage of the estrous cycle was estimated by examination of the structures present on both ovaries from each mare. Follicular phase was defined as the presence of at least one follicle ≥ 25 mm in diameter and absence of an active CL. Luteal phase was characterized by a large (≥ 25 mm), vascularized CL. When neither CL or follicle ≥ 20 mm was present, the cycle state was termed “indeterminant”. Small, dense, avascular corpora albicans may be present

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