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The effect of rivastigmine on the LPS-induced suppression of GnRH/LH secretion during the follicular phase of the estrous cycle in ewes

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ABSTRACT

This study was designed to determine the effect of a potent subcutaneously injected acetylcholinesterase inhibitor, rivastigmine (6 mg/animal), on the gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) release during inflammation induced by an intravenous lipopolysaccharide (LPS) (400 ng/kg) injection in ewes during the follicular phase of the estrous cycle. The results are expressed as the mean values from −2 to −0.5 h before and +1 to +3 h after treatment. Rivastigmine decreased the acetylcholinesterase concentration in the blood plasma from 176.9 ± 9.5 to 99.3 ± 15.1 μmol/min/ml. Endotoxin suppressed LH (5.4 ± 0.6 ng/ml) and GnRH (4.6 ± 0.4 pg/ml) release; however, the rivastigmine injection restored the LH concentration (7.8 ± 0.8 ng/ml) to the control value (7.8 ± 0.7 ng/ml) and stimulated GnRH release (7.6 ± 0.8 pg/ml) compared to the control (5.9 ± 0.4 pg/ml). Immune stress decreased expression of the GnRH gene and its receptor (GnRH-R) in the median eminence as well as LHβ and GnRH-R in the pituitary. In the case of the GnRH and LHβ genes, the suppressive effect of inflammation was negated by rivastigmine. LPS stimulated cortisol and prolactin release (71.1 ± 14.7 and 217.1 ± 8.0 ng/ml) compared to the control group (9.0 ± 5.4 and 21.3 ± 3.5 ng/ml). Rivastigmine also showed a moderating effect on cortisol and prolactin secretion (43.1 ± 13.1 and 169.7 ± 29.5 ng/ml). The present study shows that LPS-induced decreases in GnRH and LH can be reduced by the AChE inhibitor. This action of the AChE inhibitor could result from the suppression of pro-inflammatory cytokine release and the attenuation of the stress response. However, a direct stimulatory effect of ACh on GnRH/LH secretion should also be considered.

1. Introduction

Inflammation caused by bacterial infection affects the female reproductive process. An immune/inflammatory challenge decreases the concentration of luteinizing hormone (LH) in sheep (Daniel et al., 2003). LH synthesis and release from the anterior pituitary (AP) is dependent on the pattern of gonadotropin-releasing hormone (GnRH) pulsatile secretion. Factors that can affect the GnRH pulse amplitude, duration, and frequency can disturb the female ovulatory cycle (Rivier and Vale, 1990; Kalra et al.,

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1998; Karsch et al., 2002). Inflammation caused by the i.v. injection of the bacterial endotoxin lipopolysaccharide (LPS) suppressed the GnRH/LH activity in anestrous ewes (Herman and Tomaszewska-Zaremba, 2010). Inflammation during the follicular phase of the estrous cycle in ewes interrupts the preovulatory estradiol increase and delays or blocks the subsequent LH and follicle-stimulating hormone (FSH) surges (Battaglia et al., 2000). This negative effect of inflammation on the reproduction process is mediated *via* pro-inflammatory cytokines penetrating the region of the hypothalamus during immune challenges (Watanobe and Hayakawa, 2003). Interleukin (IL)-1β and tumor necrosis factor α (TNFα) may represent the major pro-inflammatory cytokines mediating the LPS-induced suppression of GnRH and LH release, whereas the role of IL-6 seems to be insignificant (Rivier and Vale, 1990; Watanobe and Hayakawa, 2003; Herman et al., 2012).

Acetylcholine (ACh) attenuates the secretion of pro-inflammatory cytokines. *In vitro* studies show that ACh acting most likely *via* the α7 nicotinic acetylcholine receptor reduces the LPS-induced release of pro-inflammatory cytokines, including IL-1β, TNFα and IL-6 (Borovikova et al., 2000). The concentration of ACh is controlled by acetylcholinesterase (AChE), a serine protease which catalyses the hydrolysis of ACh (Taylor and Radic, 1994). The pharmacological blockage of AChE activity causes a decrease in pro-inflammatory IL-1β synthesis in the blood cells and brain (Pollak et al., 2005).

The aim of the present study was to determinate the effect of the subcutaneous (s.c.) injection of rivastigmine, a potent AChE inhibitor, on GnRH/LH secretion in follicular phase of the estrous cycle of ewes during an immune/inflammatory challenge.

2. Materials and methods

2.1. Animals

The studies were performed on adult, 3-year-old Blackhead ewes during the reproductive season (September–October). All animals were in good condition, with a body condition estimated at three on a five-point scale. The animals were maintained indoors in individual pens and exposed to natural daylight. The ewes were well adapted to the experimental conditions; and always had visual contact with neighboring ewes, even during the experimental period, to prevent stress due to social isolation. The animals were fed a constant diet of commercial concentrates with hay and water available *ad libitum*.

All procedures on animals were performed with the consent of the Local Ethics Committee of the Warsaw Agriculture University.

2.1.1. Adapting animals to experimental conditions

One month prior to the experiment, stainless steel guide cannulas (1.2 mm o.d.) were implanted under stereotaxic control into the third brain ventricle of all ewes ($n = 18$) through a hole drilled in the skull (Traczyk and Przekop, 1963). The guide cannula was fixed to the skull with stainless steel screws and dental cement. The external opening to the canal was closed with a stainless steel cup. Cannula placement was confirmed by the presence of CSF during surgery. Definitive cannula placement was determined by infused dye just prior to slaughter.

After 1 month of convalescence, the stage of the estrous cycle of ewes was synchronized by the Chronogest® CR (Merck Animal Health, Boxmeer, The Netherlands) method using an intra-vaginal sponge impregnated with 20 mg of a synthetic progesterone-like hormone. All ewes had Chronogest® CR sponges placement for 14 days. Following sponge removal, the ewes received an intramuscular injection of 500 iu pregnant mare's serum gonadotropin (PMCS) (Merck Animal Health, Boxmeer, The Netherlands). The experimental procedure began 24 h following PMSG injection.

2.2. Experimental procedures

Venous catheters were implanted into the jugular vein on the day prior to the experiment. Ewes ($n = 18$) were randomly divided into three experimental groups: Group I – control ($n = 6$); Group II – LPS-treated ($n = 6$); Group III – LPS- and rivastigmine-treated ($n = 6$). In treated animals, the immune stress was induced by the i.v. injection of LPS from *Escherichia coli 055:B5* (Sigma, St. Louis, MO, USA) dissolved in saline (0.9% (w/v) NaCl) (Baxter, Deerfield, IL, USA) at a concentration of 10 mg/l into the jugular vein (400 ng/kg). Jugular blood samples (4 ml) were taken for measurement of the peripheral hormone concentration at 10 min intervals beginning 2 h before the i.v. administration of LPS or an equivalent volume of saline injection and continuing for 3 h. Blood samples were collected into heparinized tubes and immediately centrifuged for 10 min at 1000 × *g* at 4 °C. Plasma was stored at −80 °C until assayed. One half hour prior to LPS treatment animals were treated subcutaneously with saline (Groups I and II) or rivastigmine (6 mg; Group III) (Exelon®, Novartis International, Basel, Switzerland). Concomitantly, CSF samples were collected to assay the GnRH concentration at 30 min intervals beginning 2 h before and continuing 3 h after LPS/saline injection. The CSF samples (∼0.5 ml) were taken using a microinjection pump BAS Bee™ (Bioanalytical Systems Inc., USA) at 0.5 h. The samples were kept in an ice bath during sampling and stored at −80 °C until the GnRH assay was performed.

After the blood and CSF collection, the animals were immediately euthanized and the brains were rapidly removed from the skulls. Next, the AP and hypothalamic structures were dissected. The block of brain encompassing the preoptic area and hypothalamus were sectioned sagittally and dissected from both sides into three parts, *i.e.*, the preoptic area (POA), anterior hypothalamus (AHA), medial basal hypothalamus (MBH) and median eminence (ME), according to stereotaxic atlas of the sheep brain (Welento et al., 1969). Landmarks were the mammillary body, median eminence and optic chiasm. The depths of the cuts were 2–2.5 mm for MBH and 2.5–3 mm for AHA and POA. All tissues were frozen immediately