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Mink aging is associated with a reduction in ovarian hormone release and the response to FSH and ghrelin

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ABSTRACT

The endocrine mechanisms of mink ovarian hormones release and reproductive aging are poorly investigated. The aims of our study were to: (1) identify hormones produced by mink ovaries (the steroids progesterone [P] and estradiol [E], the peptide hormone oxytocin [OT], and the prostaglandin F [PGF] and prostaglandin E [PGE]); (2) examine the effect of FSH and ghrelin on the release of the hormones listed previously; and (3) understand whether these hormones can be involved in the control of mink reproductive aging, i.e., whether aging can be associated with changes (a) in the basal release of P, E, OT, PGF, or PGE and (b) their response to FSH and ghrelin. Fragments of ovaries of young (yearlings) and old (3–5 years of age) minks were cultured with and without FSH and ghrelin (0, 1, 10, or 100 ng/mL), and the release of hormones was analyzed by EIA/RIA. We found that isolated ovaries were able to release P, E, OT, PGF, and PGE, and the levels of P produced in the ovaries of old animals were lower than those produced in the ovaries of young animals, whereas the levels of other hormones did not differ. FSH was able to stimulate P and E and suppress OT and PGF and did not affect PGE release. Aging was associated with the inhibition of the effect of FSH on ovarian P and E, the appearance of the inhibitory action of FSH on OT, and the disappearance of this action on ovarian PGF. PGE was not affected by FSH, irrespective of animal age. Ghrelin was able to promote E (but not P) and suppress OT, PGF, and PGE output. Aging was associated with the appearance of an inhibitory influence of ghrelin on ovarian OT and PGE and with the disappearance of this influence on PGF output. Aging did not affect the action of ghrelin on ovarian P and E. Our observations (1) confirm the production of P and E and show that OT, PGF, and PGE are released from mink ovaries, (2) confirm the involvement of FSH and demonstrate the involvement of ghrelin in the control of mink ovarian hormone release, and (3) suggest that reproductive aging in minks is due to a reduction in basal P release and alterations in the response of E, OT, PGF (but not of PGE) to FSH and ghrelin.

1. Introduction

Human and animal aging is associated with changes in various physiological processes, including reproduction. The age-dependent reduction in fecundity can be due to alterations in the gonadotropin surge, a reduced release of ovarian steroid hormones, which promote GnRH and gonadotropin production, ovarian folliculogenesis and oogenesis, and an impaired response by the ovary to gonadotropin stimulation [1–4]. Nevertheless, the contribution of an altered ovarian response to gonadotropins in

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the development of age-related reproductive depression can vary among species. No differences were recorded in the ovarian response to FSH treatment between young adult and aged ewes [5]. Human studies have provided inconsistent results [1] although some have demonstrated an age-dependent decline in ovarian response to FSH [6]. In mice [7] and cows [2,8], FSH stimulated ovarian follicular growth in young animals to a greater extent than in old animals. Therefore, reproductive aging in some species may be induced by the reduction in both gonadotropin output and ovarian response to FSH, whereas in other species, it is caused only by reduced FSH release. Age-dependent changes in the plasma level of the hormones GH, insulin-like growth factor (IGF)-I (mice, humans [9,10]), IGF-II (mink [11]), oxytocin (OT; mice [12]; humans [13]), and prostaglandins (mice [14]) have been reported, but their involvement in the control of reproductive aging has been studied either insufficiently (GH/IGF-I axis) or not at all (IGF-II, OT, prostaglandins).

Reproductive functions are regulated not only by gonadotropins but also by other hormones [15]. Recently, the involvement of the metabolic hormone ghrelin in the control of ovarian function at the level of both the central nervous system and the ovary has been demonstrated (see review [16,17]). It can directly control the function of ovarian cells by stimulating proliferation, inhibiting apoptosis, and promoting progesterone (but not estradiol), IGF-I, and vasotocin secretion (chicken [18], pig [19–21], rabbit [22], buffalo, [23], and stimulating prostaglandin F (PGF) and prostaglandin E (PGE; pig [24]) release. The direct influence of ghrelin on the ovaries in other species has not yet been studied. Although a negative correlation between plasma ghrelin, FSH, steroid hormones, and puberty in humans has been reported [25–27], to our knowledge, the involvement of ghrelin in the control of reproductive aging has not been studied in any species.

Mink (Mustela vison) represents a good model for the study of endocrine control of reproduction and aging because these animals have numerous reproductive problems and age-dependent changes in fecundity. Aging in this species is associated with poor expression of estrous, the absence of ovulation, and high embryo mortality after repeated mating and stress, lack of ovarian stimulation after hormonal treatments and so forth. Farm breeding of these fur animals and therefore their long exploitation and reproduction are important from an economic viewpoint, but the hormonal control of mink reproduction has been studied insufficiently [28,29]. The release and the involvement of gonadotropins and ovarian steroid hormones (progesterone and estradiol) [28,30] and the metabolic hormones insulin, IGF-I, thyroid hormones [30,31], prolactin, and melatonin [28] in the control of mink reproduction have been reported. The release of other hormones (OT, prostaglandins) by mink ovaries has not been documented. The role of ghrelin in the control of mink ovarian function has not been studied. The endocrine mechanisms of mink reproductive aging remain completely unknown.

The aims of our study were to: (1) identify hormones produced by mink ovaries (the steroids progesterone [P] and estradiol [E], the peptide hormone OT, and the PGFα and E2 [PGE]); (2) examine the effect of known (FSH) and unknown (ghrelin) regulators on the release of the mink ovarian hormones listed previously; and (3) understand whether these hormones could be involved in the control of mink reproductive aging, i.e., whether aging is associated with changes in the basal release of P, E, OT, PGF, or PGE and their response to FSH and ghrelin.

2. Materials and methods

2.1. Animals

Experiments were performed at the commercial farm Liesek (Čimhova, Slovakia) on female American minks (Neovison vison) of the standard variety, housed in standard Slovak farm conditions [32,33]. Briefly, animals were housed individually in metal cages 90 × 50 × 50 cm connected with wood houses 50 × 50 × 50 cm. All female minks had visual contacts with other female minks and male minks. These outdoor cages with animals were subjected to natural seasonal changes in temperature and photoperiod. One time per day, animals were fed with standard diet based on minced chicken and beef meat with addition of cereal groats, vitamins, and minerals [33]. Animals were mated two times in March, pregnancy length varied from 35 to 70 days (due to variation of gravidity latent phase between 1 and 25 days), parturition occurred in May, and kits weaning was completed in June to July. All animals were in a good clinical and metabolic state with good fur quality. In December, pelted animals were anesthetized by intramuscular injection of xylazine (Bioveta; Ivanovice na Hane, Czech Republic, 1 mg/animal) and then killed by cardiac injection of T61 (Intervet International BV, Boxmeer, Netherlands, 500 mg/animal) in accordance with Slovak and European Union regulations on animal welfare. Two groups of animals were compared—young (yearlings) and old (3–5 years of age). Visual inspection of the ovaries showed that all females were in anestrus—ovaries did not contain follicles more than 1 mm in diameter or CLs.

2.2. Culture of ovarian fragments

Ovaries were collected immediately after pelting and transported to the laboratory in sterile physiological solution (0.9% NaCl). Two hours after collection, they were dissected into quarters by razor blade and washed three times in sterile physiological solution. Thereafter, the ovarian fragments (2–3-mg pieces) were cultured for 48 hours in Dulbecco's modified Eagle's medium at a 1:1 ratio with Ham's F-12 Nutrient Mixture and with 10% calf fetal serum and 1% antibiotic-antimycotic solution (all from Sigma, St. Louis) in Falcon 24-well plates (Becton Dickinson, Lincoln Park, NJ), one piece in 2-mL medium per well. In addition, the fragments of ovaries isolated from both young and old animals were treated with 0, 1, 10, or 100 ng/mL of porcine-purified FSH (commercially gonadotropin usually used for mink ovarian stimulation, [28,29]) or recombinant ghrelin ([Dap3]-ghrelin, PGH-3681-PI; both of biological grade; Peptides International Inc., Louisville, KY). Hormones were dissolved in culture medium immediately before the experiment. After 2 days of culture (our previous studies showed that this time is optimal to characterize