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Effect of estrogen and its antagonist on the expression of arginine vasotocin (AVT) and its oxytocic-like receptor VT3 in the shell gland of Japanese quail, *Coturnix coturnix japonica*

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ABSTRACT

Avian neurohypophysial hormone arginine vasotocin (AVT) is known to regulate shell gland contractility during oviposition. While studying the role of estrogen in the expression and regulation of AVT and its oxytocic-like receptor VT3, using *in situ* hybridization and immunohistochemistry, it was observed that the expression of AVT and its receptor was not detected in the shell gland of sexually immature Japanese quail. However, administration of estrogen to these birds not only stimulates the growth and activity (as assessed by increased mucosal fold length, total protein content and alkaline phosphatase level) of the shell gland but also upregulates the expression of AVT and VT3. Further, administration of estrogen antagonist tamoxifen to sexually mature bird shows opposite results. On the other hand, localization of *ir*-AVT, observed in the ovary of sexually mature bird, was not detected in the estrogen treated sexually immature quail. It is concluded that estrogen not only affects the growth and differentiation of avian oviduct, but also regulates the expression of shell gland AVT and its receptor VT3. Present findings suggest that the locally synthesized AVT acts in a paracrine way to upregulate VT3 receptor and thus facilitates the endocrine function of neurohypophysial AVT during oviposition.

1. Introduction

All vertebrate classes share some similarities of response to estrogenic stimulation, whereas certain aspects of avian physiology respond differently. Common responses are development of accessory and secondary sexual characteristics, control of reproductive behavior, follicular growth, and calcium regulation. Appropriate concentrations of sex steroid hormones are fundamental for the normal development and differentiation of the reproductive organs and of the central nervous system. Adult oviparous species such as birds and many fishes also require estrogen for vitellogenin synthesis, oviduct development and maturation including shell gland (avian uterus) function (Fairbrother, 2000).

Morphological differentiation, growth and synthesis of cell specific proteins occur in the oviduct of estrogenised immature chicks (Kohler et al., 1969). Estrogen regulates several hypothalamic and pituitary hormones, which in turn control ovarian functions. It has a profound influence on FSH content in the pituitary (Dunn et al., 2003). Although some studies have implicated a role for estrogen within the ovary (Goldenberg et al., 1972; Nakayama et al., 1981; Nakano et al., 1982; Gore Langton and Daniel, 1990), others have not (Spears et al., 1998).

Arginine vasotocin (AVT), the avian neurohypophysial hormone in addition to regulating fluid balance (Chaturvedi et al 1994a; Koike et al., 1977), blood pressure (Jacoby et al., 1997; Sczepanaska-Sadowska et al., 1985) and stress response (Castro et al., 1986; Romero et al., 1998) affects reproductive behavior (Grossman et al., 2002), shell contractility / oviposition (Koike et al., 1988;Rice et al., 1985; Shimada et al., 1986; Sturkey and Lin, 1966). A differential role of sex steroid is also observed in up regulating the transcription of AVT gene in the neurons of the hypothalamic paraventricular nucleus and appears to show a stimulatory role on the activity of the hypothalamo-neurohypophysial axis (AVT system) of birds. A highly significant increase in AVT gene expression as well as number of *ir*-AVT neurons and intensity of immunostaining has been observed in the PVN region of estradiol treated quail compared to control group of immature quail (Seth et al., 2004). Moreover, long day length / breeding phase that induces gonadal development (and hence increase in sex steroids) is also reported to stimulate hypothalamic vasotocinergic system in quail (Singh and Chaturvedi, 2006, 2008).

The presence of neurohypophysial peptides in the ovary was first reported in mammals. High concentrations of oxytocin (OT) are present in the corpora lutea of non-pregnant ovine and bovine ovaries, which can be a significant extra pituitary source of circulating hormone. In addition, ovarian AVP or OT may act in a paracrine manner to influence the synthesis of gonadal steroids (Wathes and

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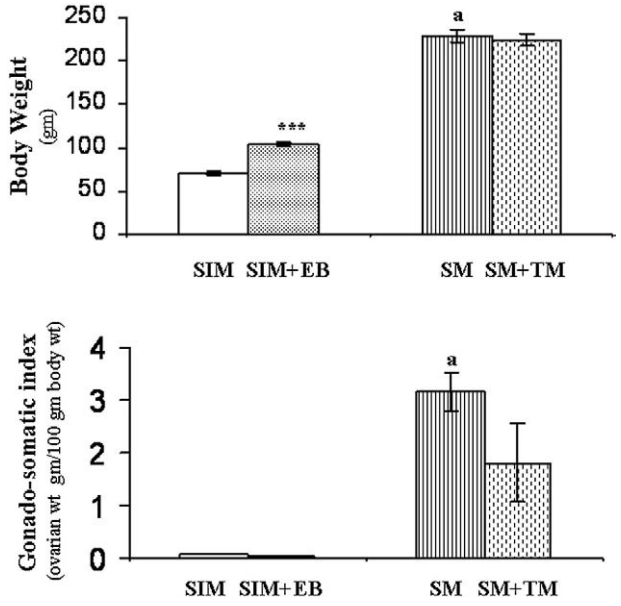

Fig. 1. Effect of estradiol benzoate (EB) and antagonist tamoxifen (TM) on the body weight and gonado-somatic index of sexually immature (SIM) and sexually mature (SM) Japanese quails respectively. Values are presented as mean±SEM. *** $p<0.001$, significance of difference from respective control, a, $p<0.001$, significance of difference from SIM.

Swan, 1982). As in mammals, the fowl ovary contains high concentrations of AVT and MT and the peptide levels vary in a different manner during the oviposition cycle (Saito et al., 1990). The estrogen and progesterone are thought to act on the uterus, because the uterine tissue contains receptors for these ovarian hormones (Kawashima et al., 1982, 1984). The slight increase in the uterine PGF concentration 6 h before oviposition may be due to the action of estrogen via its receptors and the steep increase immediately after oviposition may be caused by AVT released at the time of oviposition (Takahashi et al., 2004).

Estrogen acts by binding to the estrogen receptor that in turn, stimulates cell growth. Estrogen induces cell proliferation in the uterine and mammary gland epithelium (Martin et al., 1976; Tong and Polard, 2002). The most popular strategy to block estrogen action is to use a type of drug called anti-estrogens, which oppose the action of estrogens. Tamoxifen acts by opposing the actions of estrogens and is therefore termed as an anti-estrogen. Tamoxifen binds to the estrogen receptor protein and dislodges estrogen from the estrogen-binding pocket on the receptor. It has been shown that tamoxifen when attached to the estrogen receptor protein, stimulates the activity of certain activation proteins (AP-1) that are normally involved in cell growth (Webb et al., 1995). The estrogen receptor stimulates AP1 activity in two ways; one regulated by estrogens, the other regulated by anti-estrogens. A class of proteins called co-repressors mediates the anti-estrogen pathway. A group of drugs have been developed that have selective effects on hormone responsive tissue and therefore are known as selective estrogen receptor response modulators or SERMs (Jordan and Morrow, 1999; McDonell, 1999). The most successful of them to date is tamoxifen.

AVT and AVT gene transcripts have been reported in the ovary and shell gland of chicken (Chaturvedi et al., 1994b; Saito and Grossman, 1999). Our earlier study indicates that the expression of AVT and its oxytocic-like receptor VT3 has been upregulated in the shell gland of sexually inactive photorefractory quail following estrogen treatment while these expressions were down-regulated in photosensitive

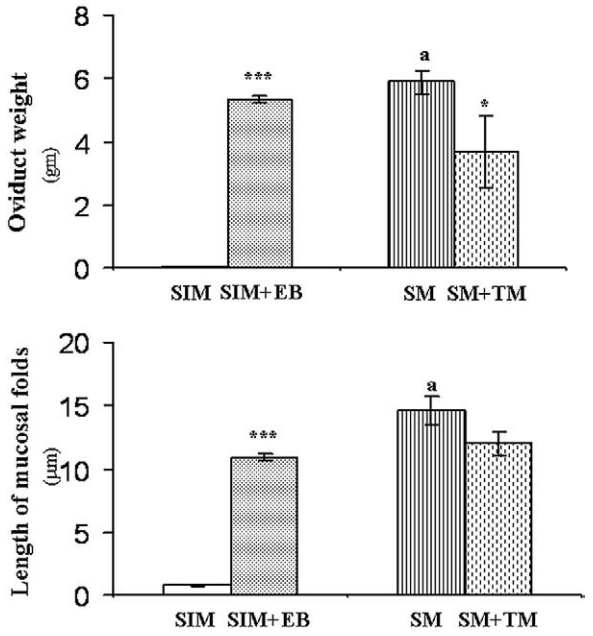

Fig. 2. Effect of estradiol benzoate (EB) and antagonist tamoxifen (TM) on the weight of oviduct and the length of mucosal fold of sexually immature (SIM) and sexually mature (SM) Japanese quails respectively. Values are presented as mean±SEM. *$p<0.05$; ***$p<0.001$, significance of difference from respective control, a, $p<0.001$, significance of difference from SIM.

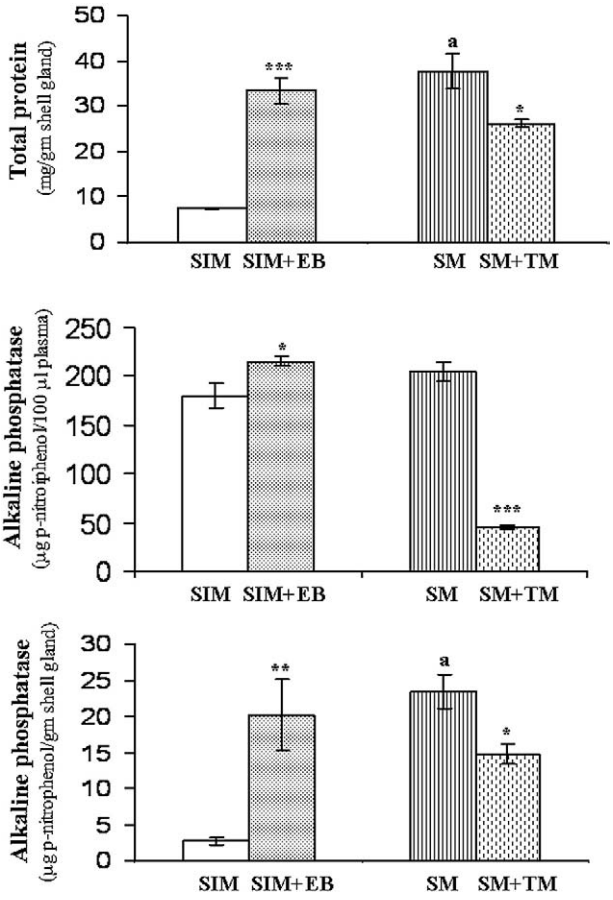

Fig. 3. Effect of estradiol benzoate (EB) and estrogen antagonist tamoxifen (TM) on the total protein and alkaline phosphatase (shell gland and plasma) in the sexually immature (SIM) and sexually mature (SM) Japanese quails respectively. Values are presented as mean±SEM. * $p<0.05$, **$p<0.01$ and *** $p<0.001$, significance of difference from respective control; a, $p<0.001$ significance of difference from SIM.