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Expression of arginine vasotocin and estrogen receptor alpha (ERα) in the shell gland altered by the specific phase relations of neural oscillations affects the reproductive physiology of Japanese quail

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HIGHLIGHTS

- Specific phase relationship of neurotransmitters alters reproductive response.
- It also alters amount of neuropeptide AVT in brain, plasma and shell gland.
- It brings about changes in the estrogen receptor alpha expression in the shell gland.

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ABSTRACT

In order to study the effect of specific phase relation of neural oscillations on reproductive regulation and the response of AVT (the avian homologue of mammalian AVP) the expression of AVT in the shell gland was monitored in sexually immature quail. In this study 3-week-old female Japanese quail were administered with serotonin precursor, 5-hydroxytryptophan followed by the dopamine precursor, L-dihydroxyphenylalanine at interval of 8 h and 12 h daily over a period of 13 days. At thirty two days post treatment, a significant decrease in gonadal activity was seen in 8 h quail although 12 h quail exhibited an increase as compared to controls. A significant decrease in plasma estradiol level was noted in 8 h quail while 12 h exhibited no significant difference compared to controls. To address the relative roles of estrogen mediated action we also investigated estrogen receptor alpha (ER-α) expression and localization in the shell gland by visualizing it through confocal immuno-fluorescence microscopy. Results indicate increased expression of immunoreactive (ir)-AVT (myometrium), ir-ER-α (epithelial cells of endometrial region), along with significant increase in hypothalamic, plasma and shell gland AVT and a rapid increase in egg laying thus maintaining full breeding condition in 12 h while low expression of ir-AVT and ir-ER-α was observed in 8 h quail along with a significant decrease in hypothalamic, plasma and shell gland AVT with the suppression of gonads thereby stopping the egg-laying behaviour was noted. These findings not only suggest the modulation of gonadal development by changing the specific phase relation of neural oscillations but also demonstrate a parallel relation of AVT and gonadal activity in both conditions. It is concluded that the egg laying performance in response to AVT is regulated by the temporal phase relationship of neurotransmitters, and in part, this effect appears to be estrogen dependent.

1. Introduction

Reproduction in birds is influenced mainly by exogenous factor as photoperiod and endogenous factors like hormones as well as neurotransmitters. The temporal phase relation of circadian neural oscillations is the basis of reproductive seasonality [1]. In addition to the external factor as the photoperiod, a specific mechanism, i.e., temporal phase relation between serotonergic and dopaminergic oscillations, is also reported to regulate reproduction in many avian species including Japanese quail (*Coturnix japonica*) [2,3]. A number of studies from our laboratory show that in general, the serotonin precursor 5-HTP and the dopamine precursor L-DOPA, if given 12 h apart i.e. 12-h relation, are gonado-stimulatory and the 8-h relation is gonado-inhibitory in different species of birds such as the Red headed bunting - [4,5]; Lal munia - [6]; Japanese quail - [7–12]; Indian weaver bird - [13]; and spotted munia - [14–16]. These phase relations are also reported to alter seasonal reproduction in different quail species such as the Jungle bush quail and the Rain Quail which breed in nature during different months of the year [17].

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In the above studies, the serotonin precursor, 5-HTP (5-hydroxy L-tryptophan) and the dopamine precursor, L-DOPA (L-dihydroxyphenylalanine) were administered at specific time intervals instead of the neurotransmitters because serotonin and dopamine cannot cross the blood brain barrier whereas their precursors do cross [18]. Further, 12-h temporal phase relations of neural oscillations alter the photo sexual responses of immature Japanese quail [19] while the 8-h relation of serotonergic and dopaminergic drugs induce reproductive regression in quail under relatively short-day lengths and exhibit scotosensitive responses under short days [20]. Specific phase relations of neural oscillations are also reported to modulate reproduction in mammals whether seasonal (Syrian hamster - [21]; Indian Palm Squirrel - [22] or continuous breeders (laboratory mice) -[23]. Thus, both photoperiod and specific temporal phase relations of neural oscillations appear to be regulators of gonadal development/activity in many avian and mammalian species.

In addition, the physiological significance of the avian neurohypophysial peptide arginine vasotocin (AVT) is connected with its well-documented effects on various reproductive behaviours [24] and stimulatory effects on shell gland contractility and oviposition [25–28]. Interestingly, AVT and its gene transcripts have been reported in the ovary and shell gland of chickens [29,30] suggesting paracrine effects of AVT on reproductive tissues. In the ovary, the amount of AVT varies during the oviposition cycle [30]. The effects of AVT are mediated by cell surface receptors that are members of the G-protein coupled receptor superfamily. Three avian AVT receptors have been cloned from chicken (*Gallus gallus*) (reviewed in [31]. The effects of AVT on shell gland contractility appear to be mediated by two AVT receptors, the VT1 and VT3 receptor subtypes [32,33]. In the chicken, expression of the "oxytocic-like" VT3 receptor appears to be confined to myometrium [33]. It has been reported that VT1 receptor mRNA levels are highest at the time of oviposition [34]. A more recent study reports that AVT and its receptor VT3 are up-regulated during gonado-stimulatory photoperiodic conditions [35,36] as well as during natural breeding conditions [37,38] and are down-regulated under short day conditions [39] as well as non-breeding conditions. Further, these studies draw our attention to the fact that AVT system is extremely sensitive to gonadal steroids. The studies also indicate that the expression of AVT and its receptor VT3R in the shell gland is up-regulated by estrogens under gonado-inhibitory conditions. Gonadal hormones are important mediators of sexual and aggressive behaviour in vertebrates. Functions of oviduct are regulated by two ovarian sex steroid hormones estrogen and progesterone [40]. In most tissues the estrogen actions are mediated by estrogen receptor α (ERα) and estrogen receptor β (ERβ). ER alpha and beta (α and β) are distributed throughout diverse tissues of the body. ERα is predominantly expressed in uterus, pituitary, hypothalamus and ovarian theca cells whereas beta is primarily in ovarian granulosa cells, lungs. The uterus is the major target tissue for estrogen and significant levels of ERα are expressed throughout all the major uterine compartments (luminal and glandular epithelium, stroma and myometrium) and the expression level varies in accordance with elevation of circulating 17β estradiol to elicit proliferative response. Evaluation of the expression and localization of these receptors is the key in clarifying the mechanism of estrogen action on cell proliferation and functional differentiation of reproductive tissues. In this study changes in the expression and localization of ERα were determined on the administration of neurotransmitter precursors at two different specific phase relations. Recent evidences suggest that the neuropeptide AVT critically mediates these gonadal hormone effects on egg-laying/reproductive behaviour [38] and have direct influence on behaviour action.

The studies performed earlier on quail by estrogen administration documenting an increase in AVT and its oxytocic-like receptor expression in the shell gland do not address possible changes in reproductively active/inactive condition due to the temporal synergism of neural oscillations. Since the studies involving the effects of interaction between the neurotransmitters on AVT are lacking hence the present study was designed to elucidate the effect of gonado-stimulatory 12-h and gonado-inhibitory 8-h phase relations of serotonergic and dopaminergic drugs on the expression of AVT in Japanese quail and to correlate with gonadal status. In addition, to address the relative roles of estrogen mediated action estrogen receptor alpha (ER-α) localization and expression in the shell gland was also studied for the first time.

2. Material and methods

Sexually immature 3-week-old female Japanese quail (*C. japonica*) were purchased from the Central Avian Research Institute, Izatnagar, Bareilly, India, and all the experiments were conducted in accordance with institutional practice and within the revised framework of animals (Scientific Procedures) Act of 2002 of Government of India on Animal welfare. Birds were fed initially with quail starter ration and at 5 weeks of age onwards were fed with quail layer mesh. Both food and water were available ad libitum. For the required photoperiodic condition, quail were maintained in light proof room fitted with tube lights (300 lx at cage floor) controlled by automatic timers.

Twenty four quail were weighed and were randomly divided into three groups ($n = 8$ each group) at 3 weeks of age. The specific phase relation of circadian serotonergic and dopaminergic activity was induced as follows according to Chaturvedi and Bhatt [41]: Table 1 shows the following details of the experimental design.

- Group I (control): Birds received normal saline (0.9% NaCl) twice daily for 13 days. (Since two normal saline injections either at 8- or 12-h intervals had a similar effect, only one control was taken into account.)
- Group II (8-h): Birds in this group were administered with serotonin precursor, 5-HTP (5-hydroxytryptophan) at 8:00 a.m. followed by administration of the dopamine precursor, L-DOPA (L-dihydroxyphenylalanine) at 4:00 p.m. establishing an 8-h relationship between two injections.
- Group III (12-h): The treatment schedule was similar to group II, however, L-DOPA was administered at 8:00 p.m. establishing a 12-h relationship.

All the injections (normal saline and 5 mg per 100 g body weight of 5-HTP and L-DOPA) were given intra-peritoneally once daily in 0.1 ml of solution for 13 days in continuous (LL) condition of light to avoid possible photoperiodic interference of a LD cycle in neuroendocrine entrainment by injections of the precursor drugs. Thereafter, all the three groups were shifted to long day length (LD 16:8) condition (lights switched on at 6:00 a.m. and switched off at 10:00 p.m.) and maintained

Table 1
Experimental Design.