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Comparative Biochemistry and Physiology, Part A 143 (2006) 202–210
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Effect of long and short photoperiod on vasotocin neurons of paraventricular nuclei and adrenal function of water deprived Japanese quail

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Received 17 July 2005; received in revised form 17 November 2005; accepted 21 November 2005
Available online 9 January 2006

Abstract

The responses of magnocellular neurons of paraventricular nuclei (PVN) and changes to adrenal activity to water deprivation in Japanese quail maintained under gonado-inhibitory and stimulatory photoperiods were examined. Water deprivation of 4 days resulted in a 12% decrease in body weight of sexually regressed short day (SD, 6L:18D) quail, while the decrease was more (18%) in sexually stimulated long day (LD, 16L:8D) quail. The increase in plasma osmolality following water deprivation was also more (47%) in LD than to SD quail (36%). Under the LD condition, quail had increased numbers, sizes and immunostaining of ir-AVT neurons of PVN compared to SD condition. A significant increase in the number of ir-AVT neurons was observed following 4 days of water deprivation in both SD and LD quail compared to their respective fully hydrated controls. However, the degree of response was more under the LD compared to the SD condition suggesting that gonado-stimulatory long days increase the activity/response of the AVT system. Increased adrenal ascorbic acid content (i.e., activity) was also observed to quail of LD when compared to SD treatment. However, osmotic stress led to adrenal hypertrophy and hyperactivity of quail of both of the photoperiodic regimes. Our findings indicate that not only osmotic stress but also photo-gonadal stimulation upregulates the expression of hypothalamic AVT genes and increases the localization of ir-AVT in many neurons of PVN. The above results support the existence of a parallel adrenal–gonad relationship and increase in adrenal function during osmotic stress, which also leads to simultaneous increase in AVT system. We conclude that photo-sexual conditions alter hypothalamic vasotocinergic and adrenal activity in Japanese quail and the degree of stimulation of the two systems following osmotic stress is higher under gonado-stimulatory LD conditions.

Keywords: Arginine vasotocin; Paraventricular nuclei; Adrenal; Water deprivation; Japanese quail; Photoperiod

1. Introduction

Among vertebrates, the most highly evolved photoperiodic mechanisms are found in birds. The stimulatory role of long days and inhibitory role of short days on the reproductive activity of temperate and tropical birds is well known (Chaturvedi and Thapliyal, 1979; Chaturvedi, 1982; Follett and Robinson, 1980; Deviche and Small, 2001). Photoperiod increases causes seasonal and unseasonal development of avian gonads and may even maintain the reproductive system in a continuous state of activity (Nicholls et al., 1983; Charles et al., 1992). In Japanese quail, short days are gonado-inhibitory and long days are stimulatory. In young Japanese quail, the daily photoperiod exerts a definite effect on gonadal development and precocious sexual development can be attained by stimulatory photoperiods (Chaturvedi et al., 1992, 1993).

In birds, arginine vasotocin (AVT), the neurohypophysial hormone produced by hypothalamic neurosecretory magnocellular neurons of supraoptic (SON) and paraventricular nuclei (PVN) are reported to be released in response to osmotic stress and oviposition (Chaturvedi et al., 2000; Seth et al., 2004a; Gubrij et al., 2005). Although magnocellular AVT neurons are found in both supraoptic (SON) and paraventricular (PVN) nuclei, that of PVN are reported to be more responsive/sensitive to osmotic stimulation compared to SON (Chaturvedi et al., 1994, 1997) here attention was focused only on PVN region. In mammals, hyperosmolality stimulates AVP mRNA accumulation that is modified by gonadal steroids (Keefe et al., 1995; Catudiac-Vallero et al., 2000). A complete disappearance of AVP innervation in brain of European hamster was found when

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1095-6433/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2005.11.022

exposed to a short photoperiod, while long photoperiod was associated with increased AVP innervation (Buijs et al., 1986). Studies in Siberian hamster reveal increase in AVP mRNA in long photoperiod than in short photoperiod (Duncan et al., 1995; Freeman et al., 2002). Studies in several fish species reveals a high plasma AVT during day than at night (Kulczykowska and Stolarski, 1996; Kulczykowska et al., 2001). Changes in VT innervation in male birds as a function of physiological and experimental manipulations of circulating levels of testosterone have also been reported (Panzica et al., 1996, 2001). It is well known that many species of birds are highly photoperiodic and their circulating testosterone (T) levels fall to very low values when males are exposed to short days. In this condition the amount of VT-ir fibres and number of cells in the lateral septum, nucleus of stria terminalis (BnST), preoptic medialis (POM) number of cells dramatically decreases both in oscerine (Voorhius et al., 1990; Deviche et al., 1996] and non-oscerine birds (Panzica, 1998; Grossmann et al., 2002). However, there have been only a few reports on the effect of photoperiod and/or gonadal steroids on AVT synthesizing magnocellular neurons of hypothalamus (review by Burbach et al., 2001; Seth et al., 2004b).

Environmental changes influence adrenal physiology more than any other endocrine glands. Environmental stimuli (e.g. photoperiod, sound, temperature) and emotional or traumatic stress regulates the activity of hyothalamo–hypophysial–adrenal axis. The changes in adrenal function following environmental stress form the basis of the theory that the adrenal plays a crucial role during periods of stress and strain. (Wingfield et al., 1995; Romero and Wingfield, 1999). Sudhakumari and Haldar (2001) reported the influence of photoperiodic alterations on the adrenal gland of two tropical birds, the spotted owlet and jungle bush quail, revealed that short photoperiod inhibited adrenocortical function and exposure to long days had a stimulatory effect. Although Japanese quail have been widely used for studying photo-sexual and other responses, the effect of day length on both vasotocinergic neurons and adrenal function has not been investigated. Here, we report the effect of short and long photoperiod, hydrated and water deprived Japanese quail on hypothalamic AVT transcript, ir-AVT neurons of PVN, adrenal and gonadal function.

2. Materials and methods

Three-weeks-old male Japanese quail (*Coturnix coturnix japonica*) (body mass 85–100 g) were purchased from Central Avian Research Institute (CARI), Izatnagar. Quail ($n=40$) were assigned into two groups and maintained under constant short (6L:18D) and long (18L:6D) day length in lightproof chambers. Birds exposed to these photoperiod conditions for 30 consecutive days were provided with food and water ad libitum. Thereafter, each photoperiod group was assigned into two subgroups of 10 quail each: control groups had free access to both food and water, and water deprived (WD) groups restricted from water, but not food for 4 days. Quail were weighed initially and after 4 days of water deprivation, after which they were anesthetized with Na-pentobarbital (3 mg/100 g body mass), and blood was collected from a wing vein into a heparinized syringe, whole blood was centrifuged and plasma was withdrawn and stored at −20 °C for analysis of plasma osmolality by freezing point osmometer (Fiske Associates, one-ten osmometer, USA).

2.1. Northern blot analysis

Anaesthetized quail ($n=5$) were sacrificed by decapitation. These brains were quickly removed, hypothalami isolated and immediately frozen in liquid nitrogen. Total RNA was isolated using Trizol reagent (Sigma-Aldrich, St. Louis, MO, USA) by the method of Chomczynski and Sacchi (1987). Hypothalamic RNA (20 μg) from each individual was subjected to electrophoresis on 1.4% w/v agarose denaturing formaldehyde gel in HEPES (*N*-[hydroxyethyl]piperazine-*N*′-[2-ethane sulphonic acid]) pH 8.0 buffer and subsequently blotted overnight by capillary transfer onto nylon membranes (Hybond N^+, Amersham) with 20× SSC as previously described (Jaccoby et al., 1997). Following transfer, membranes were hybridized overnight at 48 °C with a 209-bp *Pst*/*Eco*RI restriction fragment of cDNA encoding chicken AVT that had been random prime labeled with [α-^{32}P]dATP (BARC, Hyderabad, India) using Klenow (Roche, Indianapolis, IN, USA). The prehybridization and hybridization solutions were 250 mM NaH_2PO_4 (pH 7.4), 1 mM EDTA, 250 mM NaCl, 4% SDS, 50 μg/mL polyadenylic acid and 100 μg/mL salmon sperm DNA. Membranes were washed in 2× SSC/0.1% SDS (1× SSC contains 150 mM NaCl, 15 mM sodium citrate) three times at room temperature and one time in 65 °C. For sequential hybridization, the membranes were treated with 18S cDNA. For quantification, the densitometric volumes were calculated using a densitometer (Systronics, India). The size of the AVT gene transcript was estimated by comparison with migration of a 0.25- to 9.5-kb the RNA ladder (Sigma-Aldrich) that had been treated in parallel with RNA samples. The AVT transcript size was 0.7 kb and that of GAPDH was 2.1 kb.

2.2. Testes

Quail were dissected to expose testes. Length and width of the left testes was measured in situ with dial calipers and volume was calculated using the formula $4/3\pi ab^2$ (where $a=1/2$ of the long axis and $b=1/2$ of the short axis) as described earlier (Phillips et al., 1997). Then both testes were removed and weighed on a microprocessor based balance (Sartorius). Testes were fixed in Bouin's fluid for 24 h. Fixed tissues were washed, dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin-wax (m.p. 57–60 °C). 7 μm thick sections were cut in a rotary microtome, adhered to glass slides, deparaffinized, hydrated and were processed for routine haematoxylin and eosin stain.

2.3. Adrenals

After removing the testes, adrenals were removed quickly and weighed. The left adrenal was used for enzyme (3β-HSD) histochemistry and right adrenal for ascorbic acid estimation.