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Age and Sex Dependent Variations in the Arginine Vasotocin Gene in Response to Dehydration in the Chicken and Japanese Quail

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Rohit Seth and Chandra Mohini Chaturvedi (2004) Age and sexdependent variations in the arginine vasotocin gene in response to dehydration in the chicken and Japanese quail. *Zoological Studies* **43**(1): 86-92. The present study was conducted to investigate and compare age- and sex-dependent central and peripheral effects of osmotic stress (4 d of water deprivation) in 2 poultry species: the chicken (*Gallus gallus domesticus*) and Japanese quail (*Coturnix coturnix japonica*). Following dehydration in sexually immature (young) and mature (adult) birds of both sexes, Northern blot analysis was performed, and changes in body weight and plasma osmolality were estimated. Following 4 d of water deprivation, young and adult quail of both sexes exhibited significant losses of body weight and increases in plasma osmolality compared to their hydrated controls. Northern blot analysis revealed 1.24- and 1.95-fold increases in AVT transcripts in water-deprived adult male and female and 1.45- and 1.42-fold increases in young male and female quail, respectively; whereas 1.27- and 1.6- and 1.44- and 1.65-fold increases compared to the controls in water-deprived adult and young, male and female chickens, respectively, were found. We concluded that osmotic stress induced by water deprivation is a potent stimulator for hypothalamic gene expression in the chicken and Japanese quail, and that the response of the AVT system varies with the reproductive age and sex of the birds. In view of the remarkable difference in the amount of hypothalamic AVT transcripts and the peripheral responses of young and adult birds to osmotic stress, the present study suggests a modulatory role of sex steroids in water homeostasis.
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Key words: Sexual dimorphism, Vasotocin gene expression, Dehydration.

Arginine vasotocin (AVT) and mesotocin (MT), the avian neurohypophysial hormones, are among 16 naturally occurring neuropeptides found in vertebrates (Acher et al. 1970 1997). Both AVT and MT are reported to be synthesized by neurons of the anterior hypothalamus (Acher et al. 1970), specifically magnocellular neurons of the supraoptic (SON) and paraventricular nuclei (PVN). Water deprivation is a potent osmotic stress and has significant effects on the hypothalamic machinery regulating body homeostasis. In chickens, the plasma AVT level increases during water deprivation which provides a good correlation between plasma AVT and osmolality (Koike et al. 1977 1979, Arad and Skadhauge 1984, Arad et al. 1985, Stallone and Braun 1986). Results from *in situ* hybridization studies indicated that neurons of the PVN are more sensitive than neurons of the SON to osmotic stimulation caused either by water deprivation (Chaturvedi et al. 1994) or injection of hypertonic saline (Jaccoby et al. 1997) and saline drink (Chaturvedi et al. 1997). Furthermore, there is a gender-related difference in osmotic control of AVT release and hypothalamic AVT gene expression in adult Japanese quail (Chaturvedi et al. 2000).

Water loss from the body and parameters directly related to it, such as a decrease in body weight or an increase in plasma osmolality, depend to a large extent on the surface area of the individual. For example, evaporation needed for tolerance to desert conditions is maximum in small mammals like mice in comparison to larger mammals like camels; therefore in terms of decreases

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in body weight, the mouse is more adversely affected in comparison to the camel. Increased surface area facilitates evaporative cooling hence prevents water loss in individuals with larger surface areas in comparison to individuals with smaller surface areas. To some extent, water conservation is also related to the tolerance of animals to dehydration; for example, desert rabbits can tolerate water loss approaching 50% of their body weight; however, camels can tolerate a loss of about 27% (Folk 1974). Special adaptations are seen among some species, for example, 1) large mammals such as camels have water-conserving sweat glands; 2) in aquatic mammals such as seals and whales, the fat content of the milk is very high, which is an adaptation associated with their environment which has a low availability of fresh water; and 3) sea birds and some reptiles have salt glands (nasal glands) through which they excrete the major portion of their ingested salt. These adaptations solve the challenge of existence in severe heat and adverse climatic conditions by the judicious [release/output] of derived water from a delicate and precarious conservation system. The rhythmicity of neurohypophyseal function in domestic hens, like that in mammals is reported to be disrupted by osmotic stress (Chaturvedi et al. 2001).

Information on the effect of dehydration stress on the synthesis of AVT in young birds is almost lacking, except for a short-term dehydration study (19 to 48 h) in a 30-d-old female chick in which hypothalamic AVT gene expression was only slightly upregulated after short-term water deprivation, while the increase in plasma AVT was even greater than that reported in hens (Mühlbauer et al. 1992). In view of the above reports and i) a differential response of young and old to stressful conditions, ii) sexual dimorphism in AVT gene expression, and iii) species variations in water balance following osmotic challenge, the present investigation was undertaken to determine the age- and sex-dependent variations on hypothalamic AVT gene expression at the transcriptional level in young (sexually immature) and old (sexually mature) Japanese quail and chicken following dehydration. Associated parameters, such as body weight and plasma osmolality, were also compared.

MATERIALS AND METHODS

Three-week-old sexually immature young (65 -75 gm) and 7-wk-old sexually mature adult female (150-180 g) and male (140-155 g) Japanese quail and 2-wk-old young white leg horn chicken (110-130 g) and 6-mo-old adult female (1250-1450 g) and adult male (1350-1500 g) white leg horn chickens were housed in individual cages maintained under natural day length and exposed to ambient temperatures (35-38°C). Tap water and commercial chicken/quail ration were provided *ad libitum*. Birds of each age and sex group were individually weighed and randomly divided into 2 groups (n = 4-8). Control group birds were provided with both food and water *ad libitum*, whereas experimental group birds had free access to food but were deprived of water for 4 d prior to sacrifice. At the end of 4 d, birds of each group were weighed, then sacrificed by decapitation. The brain was quickly removed, and the hypothalamus was isolated, snap-frozen in liquid nitrogen, and stored at -70°C. Total RNA was isolated using Trizol reagent (Invitrogen/Gibco BRL, Munchen, Germany) on the basis of the method described by Chomczynski and Sacchi (1987). Fifteen micrograms of total RNA was separated on a 1.4% w/v agarose denaturing formaldehyde gel in morpholino-propane sulfonic acid (MOPS) buffer (pH 8.0) and subsequently blotted overnight by capillary transfer onto nylon membranes (Hybond N^+, Amersham, Germany), followed by UV cross-linking (150 mJ; gene linker, Bio Rad, München, Germany). The AVT-specific probe (260-bp cDNA directed towards the distal 3' glycopeptide part of the chicken AVT gene; Hamann et al. 1992) was labeled with ^{32}p dCTP for Northern blot analysis, using a random priming method (Megaprime DNA labeling system; Amersham, Braunshweig, Germany) according to Feinberg and Vogelstein (1983). Hybridization proceeded overnight at 42°C. Approximately 5 x 10^6 cpm was used per filter in 6 ml of hybridization buffer without dextran sulfate. Following washing, filters were exposed to x-ray film (Kodak, X-Omat, USA). Exposure time was 48 h at -70°C using a single intensifying screen. Filters were re-probed with GAPDH (a housekeeping gene) for normalization of the AVT gene. Plasma osmolality was measured by a freezing point osmometer, (microprocessor-controlled Fiske one-ten osmometer, MA, USA) in 20μl of plasma. For Northern blot data, densitometry (Systronics, Bangalore, India) of autoradiograms, body weight, and plasma osmolality, Student's t-test was employed to compare between control and water-deprived groups.

RESULTS

Body weight

Water deprivation (WD) for 4 d produced significant decreases in body weight in young female (21.6%, $p < 0.001$) and male Japanese quail (16.7%, $p < 0.001$) compared to their respective controls. Water-deprived adult female and male quail also showed decreases (12.7%, $p < 0.05$; and 8.4%, $p < 0.05$) in body weight compared to their respective controls (Fig. 1). As seen in quail, chickens also responded to water deprivation, and decreases in body weight following water deprivation were observed in both young female (31.4%, $p < 0.001$) and male (30.5%, $p < 0.001$) and adult female (8.58%, $p < 0.05$) and male chicken (8.14%, $p < 0.05$) (Fig. 2). When compared between young and adults of the 2 sexes, the decrease in body weight was greater in young birds of both species. When comparing adult females and adult males of Japanese quail, the decrease was greater in females ($p < 0.05$), but no significant difference was observed between young females and young males. In chicken, no significant difference was observed between young females and young males or adult females and adult males. However, when compared between young and adults of either sex, the decrease was significantly greater ($p < 0.001$) in young birds.

Plasma osmolality

In both species, plasma osmolality significantly increased after 4 d of water deprivation, irrespective of age or sex of the birds. It increased approximately 15.11% ($p < 0.001$) in young female and 20.45% ($p < 0.001$) in young male Japanese quail and 17.4% ($p < 0.001$) in adult female and 18.86% ($p < 0.001$) in adult male Japanese quail (Fig. 3). Similarly in young female chicken, the increase in plasma osmolality was 29.0% ($p < 0.001$), however in young male chickens it was

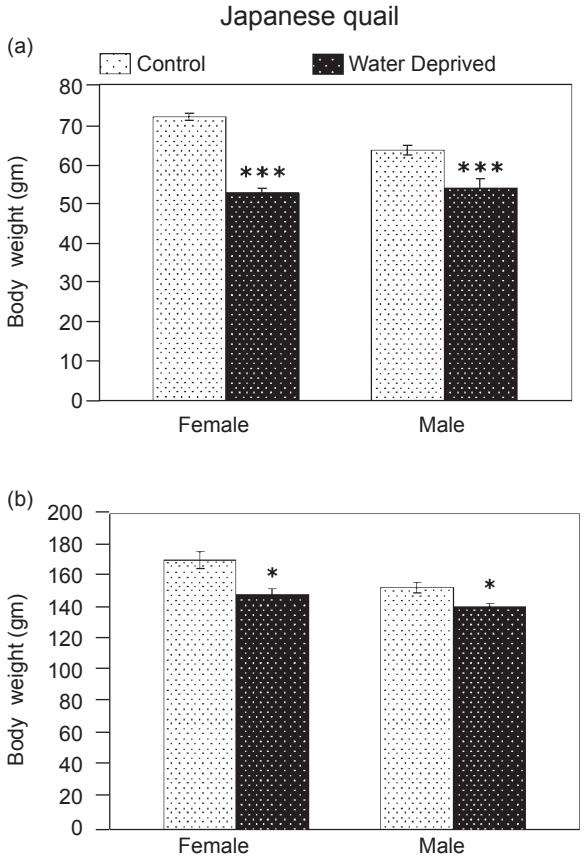

Fig. 1. Effect of 4 days water deprivation on body weight of (a) young and (b) adult quail. Values are presented as mean ± SEM. *$p < 0.05$, ***$p < 0.001$ significance of difference from control.

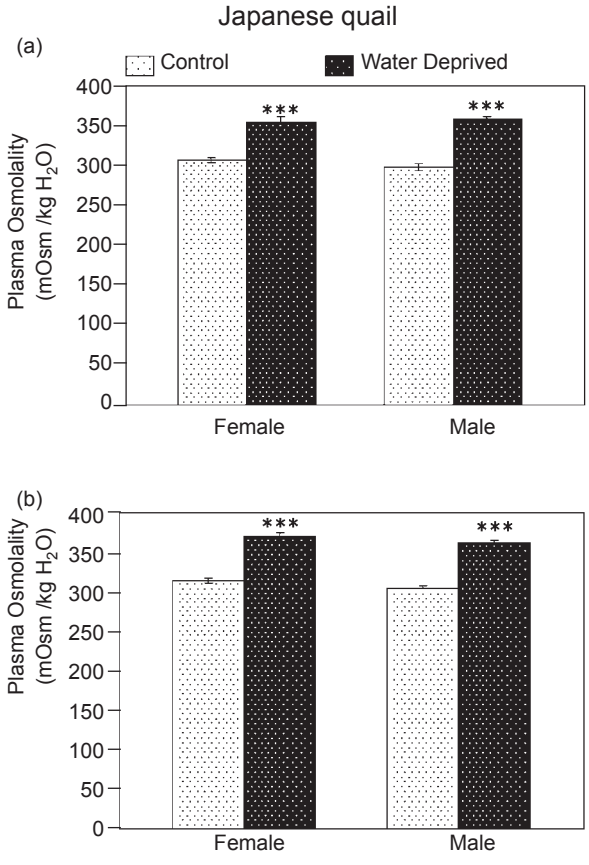

Fig. 2. Effect of 4 days water deprivation on body weight of (a) young and (b) adult chicken. Values are presented as mean ± SEM. *$p < 0.05$, ***$p < 0.001$ significance of difference from control.

23.62% ($p < 0.001$). In adult female and male chicken, the increase in plasma osmolality was 25.84% ($p < 0.001$) and 29.37% ($p < 0.001$), respectively (Fig. 4).

Northern blot analysis

Northern blot analysis indicated 1.45- and 1.42-fold increases in AVT transcripts in water-deprived young male and female quail and 1.24- and 1.95-fold increases in adult male and female quail, respectively (Figs. 5, 6); whereas 1.44- and 1.65-fold increases in water-deprived young male and female chicken and 1.27- and 1.6-fold increases in dehydrated adult male and female chicken were observed compared to their respective controls (Figs. 7, 8).

DISCUSSION

In the present investigation, we compare age- and sex-dependent responses of the AVT system in 2 poultry species, Japanese quail and chicken, following 4 d of water deprivation. This dehydration stress resulted in significant decreases in body weight ($p < 0.001$) in young and adult birds of both sexes. However, percentage decreases in body weight of females were significantly greater ($p < 0.05$) compared to their male counterparts in adult quail but not in adult chicken. Furthermore, decreases in body weight were significantly greater in sexually immature young birds of either sex in both chicken ($p < 0.001$) and quail ($p < 0.05$) compared to their adult stage, which indicates that young birds are more adversely affected by osmotic stress. In view of these differences among the 2 age groups, the sensitivity of osmotic control of the AVT system appears to be greater in adult versus young birds. Possibly for that reason, adults are more sensitive to osmotic changes and hence are able to quickly respond in order to maintain water homeostasis and thus experience fewer detrimental effects on body weight, unlike in young birds. Following 4 d of water deprivation, plasma osmolality increased in all groups of chicken and quail compared to their respective controls, but no noticeable difference or trend was observed in the percent increase of plasma osmolality in water-deprived subjects when compared between the 2 age or gender groups. Water deprivation also resulted in an increase in the amount of AVT

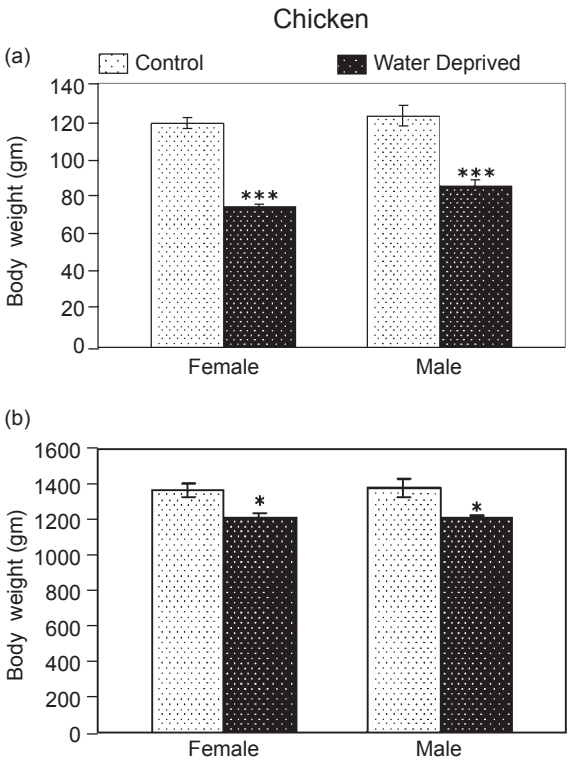

Fig. 3. Effect of 4 days water deprivation on plasma osmolality of (a) young and (b) adult quail. Values are presented as mean ± SEM. *$p < 0.05$, ***$p < 0.001$, significance of difference from control.

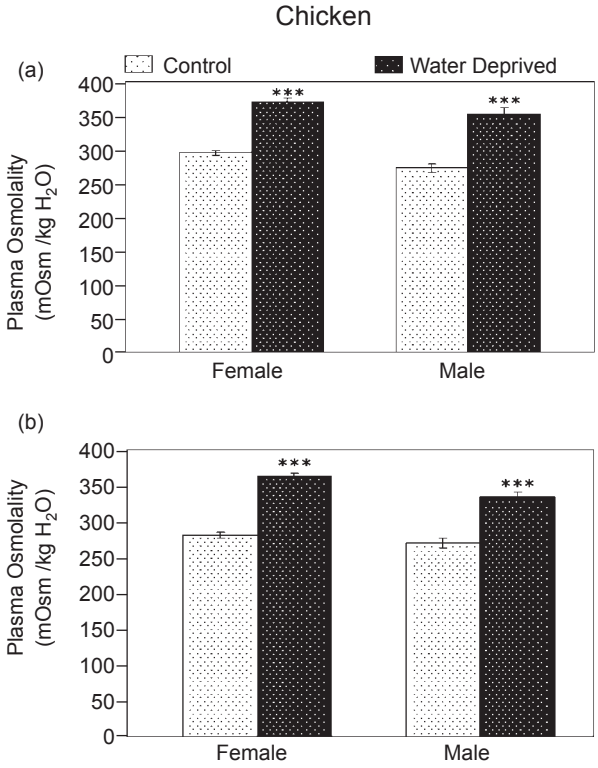

Fig. 4. Effect of 4 days water deprivation on plasma osmolality of (a) young and (b) adult chicken. Values are presented as mean ± SEM. ***$p < 0.001$, significance of difference from control.

mRNA and transcription in the hypothalamus of young and adult birds of both species and sex. Although no statistically significant increase was noted in young versus adult birds, the multiples of induction of hypothalamic AVT mRNA in water-deprived adult female quail (1.95) and chicken (1.65) were significantly greater than those of hypothalamic AVT mRNA observed in WD males of quail (1.24) and chicken (1.27). This comparison indicates both gender- and age-related differences in osmotic control by the AVT system and body responses. At the central level (AVT transcripts), differences between young and adults were not obvious, although at the peripheral level, the effect was significant (body weight decrease), suggesting that the sensitivity of the AVT system to osmotic stress is higher in adults compared to that in young quail and chicken.

Dehydration is a potent osmotic stress capable of producing both extracellular hyperosmolality and hypovolemia (Chaturvedi et al. 2000). The data show that females were more adversely affected by osmotic stress as they registered significant losses in body weight ($p < 0.05$) compared to their age-matched male counterparts. Young birds also responded to osmotic challenge by showing increased plasma osmolality and decreased body weight; however differences between the sexes were not statistically significant. These results suggest a possible role of sex steroids in the regulation of homeostasis. Not only at the peripheral level but also when compared at the transcript level, greater increases in AVT mRNA in the hypothalamus of water-deprived adult quail and chicken were seen in females ($p <$

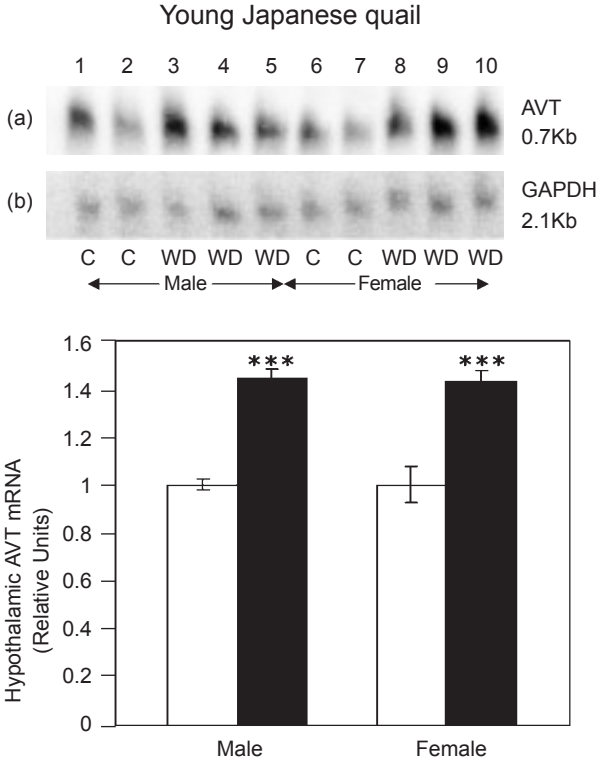

Fig. 5. Upper panel: Northern blot of hypothalamic RNA of young Japanese quail probed with fowl (a) AVT cDNA, (b) GAPDH. Each lane was loaded with 15 µg of total cellular RNA. GAPDH mRNA used to normalize AVT gene in either groups. Lower panel: Hypothalamic AVT mRNA of control □ and water deprived ■ group. Values represented as mean ± SEM. *** p <0.001, significance of difference from control.

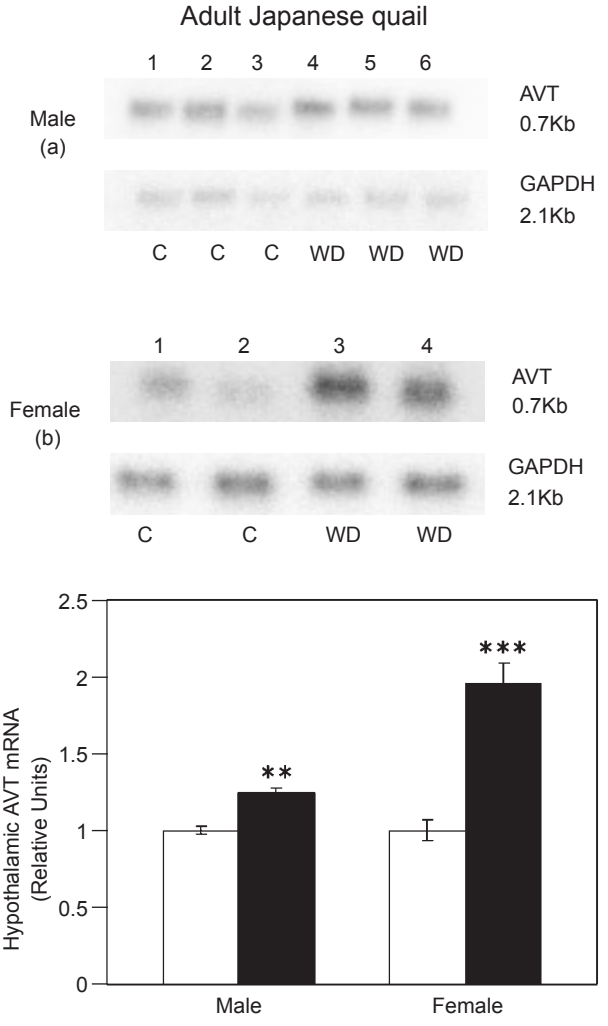

Fig. 6. Upper panel: Northern blot of hypothalamic RNA of adult (a) male and (b) female Japanese quail probed with a fowl AVT cDNA and GAPDH. Each lane was loaded with 15 µg of total cellular RNA. Lower panel: Hypothalamic AVT mRNA of control □ and water deprived ■ group. Values represented as mean ± SEM. ** $p < 0.01$, *** $p < 0.001$, significance of difference from control.

0.001) than in males ($p < 0.01$). Plasma AVT levels were reported to increase due to dehydration (Muhlbauer et al. 1992, Takeshi et al. 1998, Chaturvedi et al. 2000). Our results also suggest that females are more adversely affected by water deprivation. It seems the requirement of AVT in adult females (of both chicken and quail) is greater during dehydration, possibly due to the dual role of AVT in the regulation of water balance as well as in oviposition, and suggests gender-related differences in the osmotic control of AVT gene expression. Further studies are in progress to investigate the modulatory role of sex steroids during the response of birds to osmotic stress.

Our data suggest sexual dimorphism in the levels of hypothalamic AVT mRNA in water-deprived male and female Japanese quail. The degree of increase was greater in females than in males. However, no such difference was noted among male and female chicken, both of which showed increased AVT transcripts after water deprivation. These findings appear to be in disagreement with a report of Jurkevich et al. (1997), who found that the AVT gene transcript increased in osmotically stressed male but not in female domestic chicken. The difference may possibly have been due to the duration of water deprivation (4 d in the present study vs. 2 d in the earlier study). Different findings in quail and chicken suggest a species difference. The mechanism underlying sex differences in adult quail in response to osmotic stress may be correlated with strong sexual dimorphism in AVT-expressing neurons and reproductive behavior. The results suggest that in spite of being closely related species, the response of the 2 species to the same stimulus may differ due to their different behavior and physi-

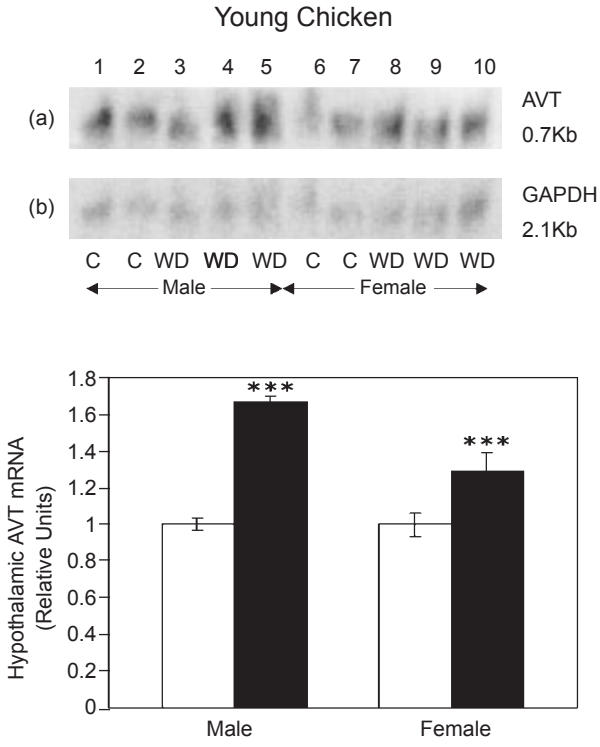

Fig. 7. Northern blot of hypothalamic RNA of young chicken probed with fowl (a) AVT cDNA and (b) GAPDH. Each lane was loaded with 15 μg of total cellular RNA. Lower panel: Hypothalamic AVT mRNA of control □ and water deprived ■ group. Values represented as mean ± SEM. ***$p < 0.001$, significance of difference from control.

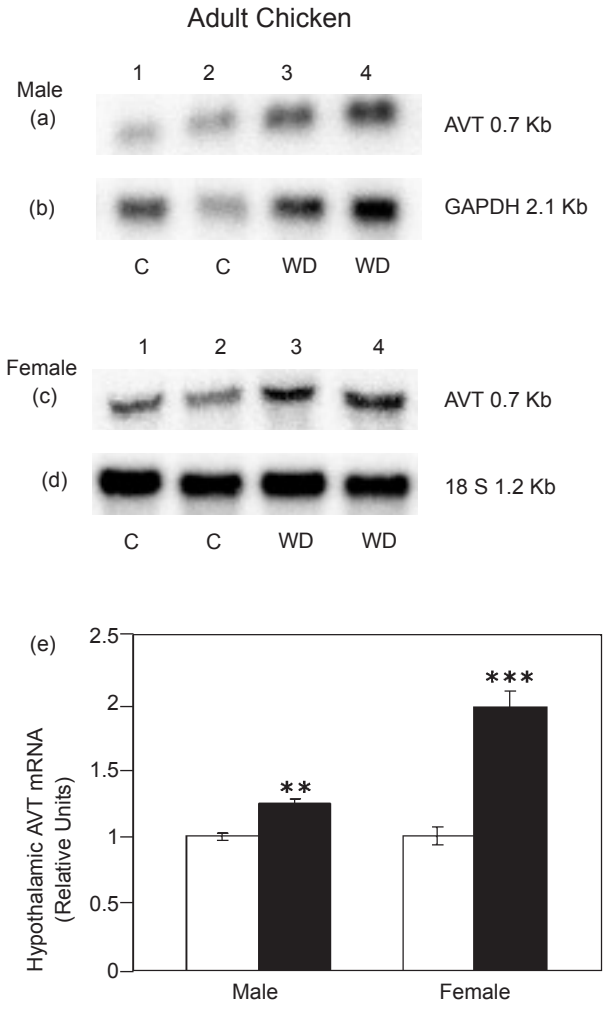

Fig. 8. Northern blot of hypothalamic RNA of adult chicken probed with AVT cDNA (a & c), GAPDH (b) and 18S (d). Each lane was loaded with 15 μg of total cellular RNA. GAPDH and 18S was used to normalize AVT gene in male and female group respectively. Lower panel: Hypothalamic AVT mRNA of control □ and water deprived ■ group. Values represented as mean ± SEM. **$p < 0.01$, ***$p < 0.001$, significance of difference from control.

ology. Thus remarkable differences in the response of hypothalamic AVT systems of sexually immature and mature chicken and quail to osmotic stress, and differences in the amount of hypothalamic AVT transcripts among the 2 sexes of adult birds following osmotic stress, suggest the role of sex steroids in water homeostasis.

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