Gene Expression of Arginine Vasotocin in Ovarian and Uterine Tissues of the Chicken

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ABSTRACT : The hypothalamus is the classic site of synthesis of arginine vasotocin as neurohypophyseal hormone in the chicken. However, high concentrations of arginine vasotocin were also measured in ovarian tissues by radioimmunoassay. At first. we observed specific positive signal of mRNA encoding AVT in the hypothalamus by Northern hybridization. However, we could not find any specific bands in ovarian and uterine tissues. For evidence of transcription of the arginine vasotocin gene in gonadal tissues of the chicken, this study has applied the polymerase chain reaction as a highly sensitive assay. The hypothalamus, the four largest preovulatory ovarian follicles and the shell gland (uterus) were collected at 4 h and 20 h before oviposition. The ovarian follicular tissues were separated into granulosa theca interns and theca externa layers. The uterine tissues were separated into myometrium and endometrium The extracted mRNA was converted to cDNA by reverse-transcriptase using oligo-d$(T)_{15}$ primer. Then, the cDNA was amplified by Vent polymerase and arginine vasotocin specific primers. The amplification reaction was incubated by 30 cycles successively, 95℃, 55℃ and 72℃ earth for 1 min. The comparisons of the mRNA levels encoding arginine vasotocin between the tissues were determined by semi-quantification methods. After amplification of the cDNA, the PCR products were detected in hypothalamus, ovarian tissues and uterine tissues. The results of semi-quantification showed that the levels of arginine vasotocin mRNA in ovarian iud uterine tissues were about from 1/50 to 1/1000 when compared to that in the hypothalamus. The very low levels of mRNA encoding arginine vasotocin in ovarian and uterine tissues probably led us to conclude that arginine vasotocin may play a role of local mediate acting autocrine and/or paracrine. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 695-701*)

Key Words : Chicken, Ovary, Uterus, Arginine Vasotocin, RT-PCR

INTRODUCTION

The avian neurohypophyseal peptides are synthesized in hypothalamic nuclei, SON and PVN, and are axonally transported to the neurohypophysis (Panzica, 1985; Tennyson et al., 1985). In mammals, there are several reports that neurohypophyseal peptides are also present in the ovaries. Data obtained from radioimmunoassay (RIA) (Watkin and Choy, 1988), high performance liquid chromatography (HPLC) (Flint and Sheldrick 1983; Writhes et al., 1982) and fast-atom bombardment mass spectrometry (Fint and Sheldrick, 1986) also indicate that the ovarian and hypothalamic neurohypophyseal peptides are similar substances. Also. bovine luteal oxytocin mRNA was identified using a bovine hypothalamic oxytocin cDNA as a probe (Ivell and Richter, 1984). Therefore, these reports suggest that gonadal tissues are able to synthesis oxytocin and vasopressin.

In the chicken, arginine vasotocin (AVT) and mesotocin (MT) was found in the extracts of ovaries and raptured follicles by HPLC and RIA (Watkin and Coys. 1988). Also, the inhibition curves with serial dilution of extracts of chicken theca and granulosa layers of the ovarian follicle, when assayed in specific AVT and MF RIAs. were parallel with the hormone standard (Saito et al., 1990).

Recently, the mRNA encoding AVT was cloned from a chicken hypothalamic library and this nucleotides sequence was determined (Hamann et al., 1992). It has been reported that mRNA encoding AVT was also identified in ovarian and uterine tissues by RT-PCR (Chaturvedi et al., 1994). However. in the later study neither the sampling time during oviposition cycle were recorded nor were the different ovarian and uterine tissues separated into three and two layers, respectively. In the present study, we describe the tissue specific gene expression in ovarian and uterine tissues during oviposition cycle and discuss the possible functions of ovarian and uterine AVT.

MATERIAL AND METHODS

Animal

Ten White Leghorn hens (LSL, Lohman, Germany), about 12 months old, were used in the experiment. They were kept in individual cages under a schedule light program of 14 h and 10 h dark with food and water ad libitum. All hens had a regular laying cycle with more than 7 consecutive days. The oviposition

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times were checked every hour visually for 2 weeks before sampling.

Tissues

All tissues, the hypothalamus, the four largest preovulatory follicles and the shell gland (uterus) were removed at 4 h and 20 h before oviposition. The separation of the granulosa layer and theca interna and externa layers and the separation of the myometrium and the endometrium of the uterus were performed as previously reported method, respectively (Olson and Hertelendy, 1983; Peter et al., 1989). These tissues were immediately frozen by dry-ice and kept at -70℃ until the extraction of total RNA.

RNA extraction

The total RNA was extracted by the previous method (Chomzynski and Sacchi, 1987). Briefly, the tissues were homogenized in guanidine thiocyanate solution and the total RNA was isolated from homogenate by phenol-chloroform centrifugation. The resulting pellets of total RNA was dissolved in water, the quantification and the quality of total RNA were measured by a spectrophotometer at 260 nm.

Northern blotting analysis

Twenty μg of total RNA was fractionated by the electrophoresis in 1.2 % formaldehyde-agarose gel with 60 V constant voltage. The electrophoresed total RNA was transfer onto Hybond-N$^+$ (Amersham, Brawnshweig, Germany) by capillary transfer.

Reverse transcription

Ten μg of total RNA was converted in single stand cDNA (ss-cDNA). Ten μg of total RNA and 2 μl oligo-d(T)$_{15}$ (100 ng/μl) was diluted in distilled water in a total volume of 12.5 μl and denatured at 95℃ for 5 min. The following components were added in order to give a total reaction volume of 20 μl: 2 μl 10×reaction buffer (500 mM Tris-HCl, pH 8.3, 80 mM $MgCl_2$, 100 mM dithiothreitol), 4 μl dNTPs mix (2.5 nmol/μl each of dATP, dCTP, dGTP and dTTP), 0.8 μl M-MuLV reverse transcriptase (25 U/μl, New England Biolabs, Schalbach, Germany), and 0.5 μl human placenta RNase inhibitor (40 U/μl, Boehringer, Mannheim, Germany). The reaction mixture was incubated at 37℃ for 1 h.

PCR

Seven μl ss-cDNA solution, prepared as described above, was used for PCR amplification in a total volume of 10 μl. The PCR assay mixture contained the following components: 74.5 μl water, 10 μl reaction buffer, 4 μl dNTPs mix, 2 μl 5' primer (100 ng/μl), 2 μl 3' primer (100 ng/μl) and 0.5 μl Vent DNA polymerase (2 U/μl, New England Biolabs, Schalbach, Germany). All subsequent reaction steps were performed using a programmable thermocycler (Trio-Thermoblock, Biometra, Cottingen, Germany). The reaction was incubated for 30 cycles of 95℃, 55℃ and 72℃ each for 1 min, with elongated step of 5 min at the first 95℃ and the final 72℃. After removal of the mineral oil by extraction with chloroform, the reaction solution was stored at 4℃ until electrophoresis.

The used oligonucleotide primers 5'-primer, CTGGGTGATACAGCCTTGCGGCA, and 3'-primer, GGCGTCCATGGCACATGTGTCA, kindly provide by Dr. R. Ivell (Institute for Hormone and Fertility Research. Hamburg. Germany). These are equivalent to nucleotides 140-162 (5'-primer) and 364-385 (3'-primer) of the chicken AVT cDNA (Hamann et al., 1992) (figure 1A).

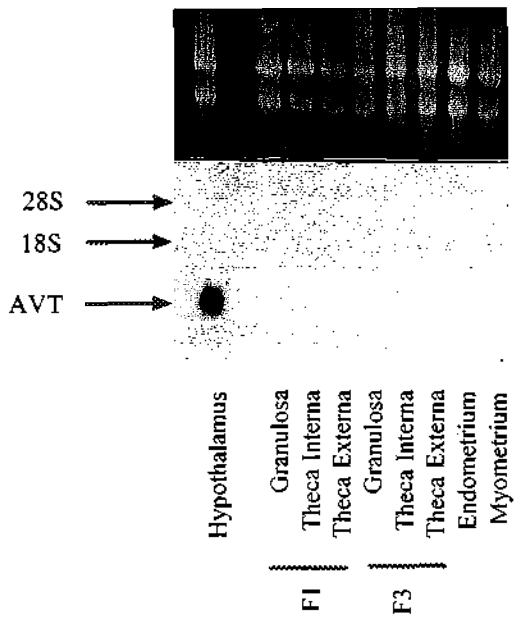

Figure 1. Northern blot hybridization of chicken hypothalamus and ovarian and uterine tissues. Upper panel is ethidium bromide staining. Lower panel is the autoradiogram of the Northern blot hybridization. Twenty μg total RNA was used for the electrophoresis. The autoradiogram was exposed for 3 days.

The PCR products were electrophoresed in 1.2 % agarose gels in 1×TAE buffer, and was transferred onto nylon membrane (Hybond-N$^+$. Anersham, Braunschweig, Germany) by capillary blotting with 0.4 N NaOH solution.

Hybridization

As chicken AVT mRNA specific probe, an AVT cDNA insert containing of nucleotides 1-359 was used

for labeling. For hybridization, the probe was labeled by random prime labeling method (Megaprime DNA labeling system, Amersham, Braunschweig, Germany) with [^{32}P]-dCTP. For hybridization, the radioactive concentration of the probe was 1×10^6 cpm/μl in final hybridization solution. Prehybridization and hybridization were performed in hybridization buffer (45% formamide, 5×SSC, 1×Denhardt's solution, 25 mg/μl sheared and denatured salmon sperm DNA and 10% dextran sulfate) at 42℃ for 2 h and 18 h, respectively. Then, the filters were washed three times with the final condition of 0.1×SSC-0.1% SDS at 60℃ for 15 min, and exposured to X-ray film (Hyperfilm-MP, Amersham, Brawnschweig, Germany) for 1 or 2 days.

Semi-quantitative PCR assay

For the determination of the relative concentration of AVT mRNA in each tissue, the semi-quantification PCR method suggested by Ivell et al. (1992) was performed. The component of the PCR reaction was same as the described above. The incubation cycle was 30 cycles for hypothalamic tissue and 39 cycles for ovarian and uterine tissues. At regular cycle intervals 10 μl samples were collected during the annealing step. These samples were blotted onto nylon membrane with dot-blot. The transferred membranes were hybridized as described above. Autoradiographs were scanned by a densitometer (Hoefer Instruments, CA, USA). For all scanned data ED_{50} or PCR cycle were calculated by the ALLFIT (De Lean et al., 1978) program for each sample (figure 3B). On the assumption thats one PCR cycle doubled the mRNA level, the relative levels of mRNA in ovarian and uterine tissues were calculated with based on ED_{50} of the hypothalamus. Semi-quantification analysis were repeated in at least two times in all samples from reverse transcription. The mean±SEM was obtained from 3 to 5 tissues.

For confirmation of the PCR product size. after terminated PCR amplification 5 μl solution was analyzed by Southern blot hybridization as previously described. For standardization between the samples, 5 μl ss-cDNA was used for dot-blot hybridization using a human β-actin (Clontech, Palo Alto, CA) probe.

Statistical analysis

All values are mean±SEM. Semi-quantification data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range test.

RESULTS

Northern hybridization

The specific positive signal of mRNA encoding AVT in the hypothalamus was observed at 700 bp by Northern hybridization. However, in ovarian and uterine tissues, any specific bands were not found (figure 1). The autoradiograph of figure 1 was exposured for 3 days. and when even the exposure of autoradiograph was 14 days, there were no bands in ovarian and uterine tissues.

PCR

After amplification of hypothalamic ss-cDNA using specific primer for chicken AVT, PCR products were observed at 246 bp (figure 2). The same size of PCR product was also observed by using in the reversed ovarian and uterine ss-cDNA from the extracted total RNA. The PCR product from the hypothalamus shown a very strong positive signal, while ovarian and uterine PCR products were week signal bands. In the granulosa layer of the F4 follicle, myometrium and endometrium. it was difficult to see the positive band after 1 day exposure, but after 2 days exposure, the same size bands were also shown in these tissues. In no sample and no primer, there were no positive bands even after 2 days exposure.

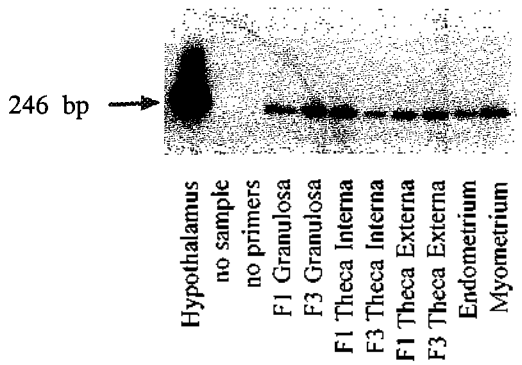

Figure 2. Autoradiogram of the Southern bolt hybridization of PCR products derived from chicken hypothalamus and ovarian and uterine tissues, correspondence to mRNA encoding AVT. The extracted total RNAs were reverse-transcripted, and amplified by PCR, 30 cycles of 95℃, 55℃ and 72℃ each for 1 min. The PCR products were electrophoresed in 1.2% agarose gels in 1×TAE buffer, and was transferred onto nylon membrane by capillary blotting with 0.4N NaOH solution.

Semi-quantification

As shown in figure 3, each autoradiograph of dot blot hybridization in earth tissue became stronger positive signals by the increased cycle. The hypothalamus sample was too strong signal compared to ovarian and uterine tissues. And then, for the semi-quantification analysis. the amplification cycle was used for 30 cycles for hypothalamic tissue and 39 cycles for ovarian and uterine tissues. We decided the

30 and 39 cycles as an adequate cycle for hypothalamus and ovarian and uterine tissues, respectively, from our preliminary experiment Semi-quantification analysis showed that the ED_{50} of hypothalamus AVT mRNA was 21.9±0.89 cycle at -4 h and 21.9 ± 0.68 cycle at -20 h before oviposition. Semi-quantification data were expressed by the ration compared to hypothalamus AVT mRNA levels as the standard. There were no significant differences of the slopes of ALLFIT curves between all tissues.

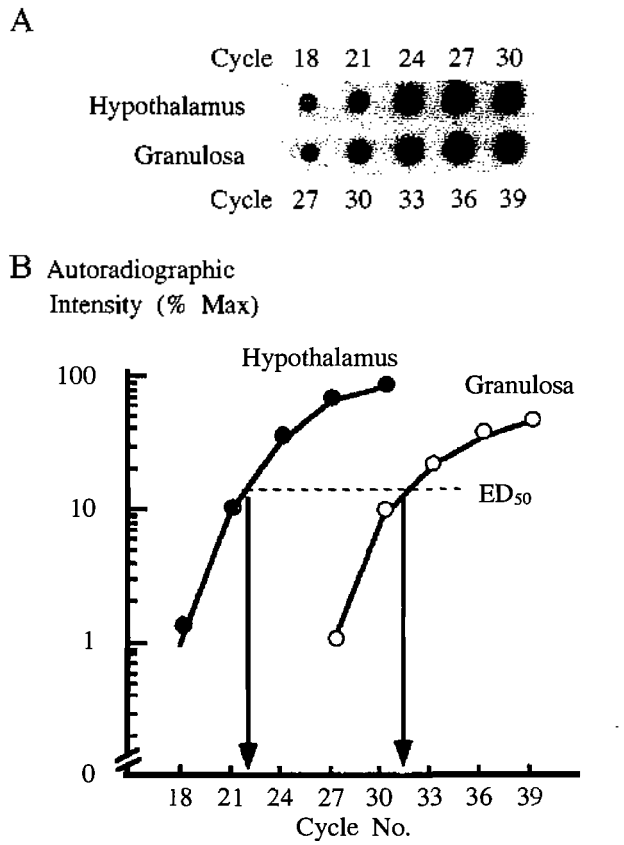

Figure 3. (A), Autoradiogram of dot blot hybridization of PCR products collected from cycles 18, 21, 24, 27 and 30 (left to right) of chicken hypothalamus and from cycles 27, 30, 33, 36 and 39 (left to right) of chicken ovarian and uterine tissues. (B), Semiquantitative analysis of PCR products derived from chicken hypothalamus and granulosa tissues. Autoradiograms were densitometrically quantified by computer image analysis. ED_{50} was calculated using ALLFIT program.

Ovaiy (Figure 4)

Ovarian and uterine AVT gene expression were about 50 to several hundreds times lower than those in the hypothalamus. In the granulosa cell layer, levels of mRNA encoding AVT from the fourth (F4) to the second (F2) largest proovulatory follicle were about 1/50 lower than in the hypolhalamus. From -28 h (= -4 h of the F2 follicle), levels of mRNA encoding AVT gradually decreased until the largest preovulatory (Fl) follicle. Levels of mRNA encoding AVT at -4 h of the Fl follicle were 1/300 lower than in the hypothalamus, and significantly lower compared to levels of mRNA encoding AVT during -44 h (= -20 h of the F2 follicle) and -92 h (= -20 h of the F4 follicle).

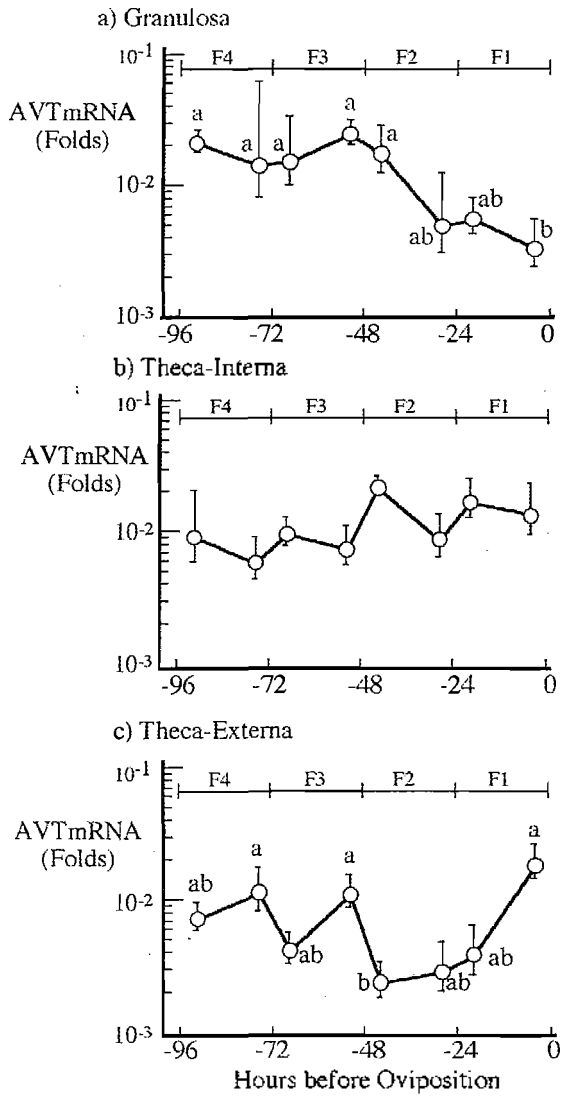

Figure 4. Relative levels of semi-quantitative PCR analysis for mRNA encoding AVT in ovarian tissues during oviposition cycle. The relative values were based on the hypothalamus AVT mRNA level as 1. F4, F3, F2 and Fl are the fourth-largest third-largest, second-largest and largest preovulatory follicle. respectively. Different letters indicate significant differences at $p<0.05$. Each point represents the mean and SEM. N=3-5.

In the theca interna layer, levels of mRNA encoding AVT were relatively constant from the F4 to Fl and were 1/50 to 1/200 when compared to those in the hypothalamus, and they also showed the tendency to increase with follicular growth. In the theca externa cell layer, levels of mRNA encoding AVT of the third largest preovulatory (F3) and F4 follicles were 1/89 and 1/242 lower than those in the hypothalamus. Levels of mRNA encoding AVT at -44 h (= -20 h of the F2 follicle) decreased to 1/423 lower and kept at low this low level until -20 h (= -20 h of the Fl follicle). At -4 h of the Fl follicle, levels of mRNA encoding AVT sharply increased about 10 times compared to -20 h of the Fl follicle.

Uterus (Figure 5)

In the endometrium, levels of mRNA encoding AVT did not changed between -20 and -4 h, and were about 1/65 lower than those in the hypothalamus. On the other hand, levels of mRNA encoding AVT in the myometrium at -20 h were 1/592 lower than those in the hypothalamus, and were also significantly lower than in the endometrium. However. at -4 h these levels increased to 1/236 lower compared to hypothalamic levels of mRNA encoding AVT.

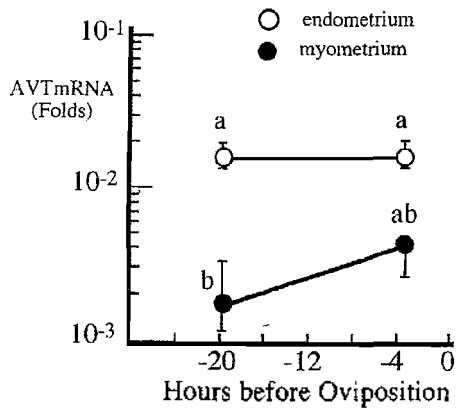

Figure 5. Relative levels of semi-quantitative PCR analysis for mRNA encoding AVT in uterine tissues during oviposition cycle. Letters and abbreviations are the same as described in figure 4.

DISCUSSION

The present data clearly demonstrated that there are large variations in levels of mRNA encoding AVT in ovarian and uterine tissues during oviposition cycle of the chicken. After amplification, PCR products from ovarian and uterine mRNA were the same size to the PCR product deprived from hypothalamic mRNA encoding AVT. 5' primer and 3' primer were designed to hybridize to exon 1 and exon 2 and 3, respectively. Therefore, the PCR product cover all three exons 1 to 3 of the AVT mRNA (Hamann et al., 1992). Thus, these data suggest that ovarian and uterine mRNA encoding AVT are identical to hypothalamic mRNA encoding AVT. Also, ovarian and uterine levels of mRNA encoding AVT were about 1/50 to1/300 lower than those of the hypothalamus, which confirms earlier data (Chaturvedi et at., 1994).

In ovarian tissue. there are some reports in mammals that oxytocin may play an role in controlling ovarian steroidogenesis (Tan et al., 1982) and uterine prostaglandin production (Flint et al., 1986; McCracken et al., 1984). Also, it has been reported that oxytocin mRNA increase in relation to ovulation (Ivell et al., 1985). In the chicken, ovarian tissues also synthesize a large amounts of steroid hormones and prostaglandins like manunal and these levels varied with follicular growth. But the actions of AVT in the chicken ovary have not been investigated. The present study showed that the variation of levels of mRNA encoding AVT during follicular growth were different between granulosa, theca interna and theca externa cell layers. In granulosa cell layer, levels of mRNA encoding AVT decreased with follicular growth. but in theca interna cell layer, levels of mRNA encoding AVT showed the tendency to increase with follicular growth and, unlike in theca exerna cell layers. these levels showed a large variation with follicular growth. These data suggest that AVT gene expression are independently regulated in each ovarian tissue, and AVT may have differential physiological action in each of these tissues.

The present study found the AVT mRNA in all three layers of ovarian follicles, but Chaturvedi et al. (1994) did not find the signal from ovarian granulosa tissue. We do not have clear explanation about this discrepancy. One possibility that AVT mRNA levels in granulosa decreased according the follicular maturation. We do not know that which preovulatory follicle they used for their experiments, when they used the largest preovulatory follicle, AVT mRNA levels in granulosa tissue of the largest preovulatory follicle were relatively lower than other tissues.

There is one report that AVT may contract the ovarian wall at the time of ovulation (Yoshimura et al., 1983). The AVT gene expression in the theca externa cell layer of the Fl follicle increased at 4 h before oviposition. These results suggest that AVT in theca externa cells may contract the ovarian wall in relation to ovulation. There are no reports about AVT specific binding sites in ovarian tissues. Yoshimura et al. (1983) suggest that there are specific AVT receptors within ovarian tissue, but they do not indicate in which tissue the receptor may be localized.

It is well known that the concentrations of AVT in the peripheral plasma increases at the time of oviposition and that this peptide participates in

regulating oviposition (Arad and Skadhauge, 1984; Nouwen et al., 1984; Tanaka et al., 1984). Also, there are some reports that ovarian follicles contain ovarian oviposition-inducing factor (OOIF) (Tanaka, 1976; Tanaka and Goto, 1976). There is the possibility that ovarian AVT may be involved in the regulation of oviposition. It was reported that the theca cell layers of the three largest preovulatory follicles and of the three ruptured follicles contained similar amounts of AVT (0.8 to 2.5 ng/tissue) (Saito et al., 1990). The AVT content of the theta layer changed significantly during the oviposition cycle and was lowest 2 h before the expected oviposition. The AVT content in the granulosa cell layer were constant (about 1 ng/tissue) and remained unchanged dunng the oviposition cycle. However, the concentration of AVT in the ovarian follicular venues plasma dose not increase at oviposition and is not higher than that in peripheral plasma (Shimada et al., 1987). Even though ovarian tissues have high levels of AVT, the present study also suggests that gene expressions of AVT in ovarian tissues are very low levels. Together wilh all data suggests that ovarian AVT dose not directly affect uterine contractility in relation to oviposition.

In mammals, there are some reports that the uterine tissue contain high level of oxytocin mRNA. and that gene expression may be related to the estrous cycle and parturition (Lefebvre et al., 1992; 1994a,b). In the chicken, there are no reports about AVT gene expression and peptide contents in the myometrium. The present study shows for the first time the increase of levels of mRNA encoding AVT in the myometrium 4 h before oviposition. On the other hand, in the endometrium levels of mRNA encoding AVT were much higher than in the myometrium, but these levels did not change during oviposition cycle. The levels of mRNA encoding AVT in myometrium were very low compared to the hypothalamus, but it is still possible that myometrium AVT may effect uterine contraction in an autocrine and/or paracrine manner

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REFERENCES

Arad, Z. and E. Skadhauge. 1984. Plasma hormone (arginine vasotocin, prolactin, aldosterone and corticosterone) in relation to hydration state, NaCl intake, and egg laying. J. Exp. Zool. 232:707-714.

Chaturvedi, C. M, Z. Zheng, T. I. Koike and L. E. Cornett. 1994. Arginine vasotocin gene expression in neuro-endocrine, reproductive and gastrointestin at tissues of the domestic fowl: detection by reverse transcriptase chain reaction. Neurosci. Lett. 178:247-250.

Chomzynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochen. 162:156-159.

De Lean, A., P. J. Munson and D. Rodbard. 1978. Simultaneous analysis of families of sigmoidal curves: application to bioasay, radioligand assay, and physiological does-response curves. Am. J. Physiol. 235:E97-E102.

Flint, A. P. F. and E. L. J. 1986. Ovarian oxytocin and the maternal recognition of pregnancy. J. Reprod. Fert. 76:831-839.

Hamann, D., N. Hunt and R. Ivell. 1992. The chicken vasotocin gene. J. Neuroendocrinol. 4:505-513.

Ivell, R., K. H. Brackett, M. J. Fields and D. Richter. 1985. Ovulation triggers oxytocin gene expression in the bovine ovary. FEBS Lett. 190:263-267.

Ivell, R. and D. Richter. 1984. The gene for the hypothalamic peptide hormone oxytocin is highly expressed in the bovine corpus luteum : biosynthesis, structure and sequence analysis. EMBO J. 3:2351-2354.

Ivell, R., N. Hunt, M. Hardy, H. Nicholson and B. Pickering. 1992. Vasopressin biosynthesis in rodent leydig cells. Mol. Cell. Endocrinol. 89:59-66.

Lefebvre, D. L., A. Giaid, H. Bennett, R. Lariviere and H. H. Zingg. 1994a. Uterine oxytocin gene expression. II. Induction by exogenous steroid administration. Endocrinology. 134:2562-2566.

Lefebvre, D. L., R. Farookhi, A. Larcher, J. Neculcea and H. H. Zing. 1994b. Uterine oxytocin gene expression. I. Induction during psedopregnancy and the estrous cycle. Endocrinology. 134:2556-2561.

McCracken, J. A., W. Schram and W. C. Okulicz. 1984. Hormone receptor control of pulsatile secretion of PGF2a from the ovine uterun during luteolysis and its abrogation in early pregnancy. Anim. Reprod. Sci. 7:31-55.

Nouwen, E. J., E. Decuypere, E. R. Khün, H. Michels, T. Hall and A. Chadwick. 1984. Effect of dehydration, homorrhage and oviposition on serum concentrations of vasotocin, mesotocin and prolactin in the chicken. J. Endocrinol. 102:345-351.

Olson, D. M. and F. Hertelendy. 1983. Avian shell gland contractility: interaction of PGF2 α and arginine vasotocin with Ca^{2+}. Am. J. Physiol. 244:C150-C157.

Panzica, G. C. 1985. Vasotocin-immunoreactive elements and neuronal topology in the suprachiasmatic nucleus of the chicken and Japanese quail. Cell Tissue Res. 242:371-376.

Porter, T. E., B. M. Hargis and J. L. Silby, M. E. El Halawani. 1989. Differential steriod production between theca interna and theca externa cells: a three-cell model for follicular steroidogensis in avian species. Endocrinology. 125:109-116.

Saito, N., S. Kinzler and T. I. Koike. 1990. Arginine vasotocin and mesotocin levels in theca and granulosa layers of the ovary during the oviposition cycle in hens (Gallus domesticus). Gen. Comp. endocrinol. 79:51-63.

Shimada, K., N. Saito, K. Itogawa and T. I. Koike. 1987. Changes in plasma concentrations of arginine vasotocin

and prostaglandin F after intrauterine injections of prostaglandin $F_2\alpha$ and acetyl choline at various times during oviposition cycle of the domestic fowl (Gallus domesticus). J. Reprod. Fert. 80:143-150.

Tan, G. J. S., R. Tweedale and J. S. G. Biggs. 1982. Effects of oxytocin on the bovine corpus luteum of early pregnancy. J. Reprod. Fert. 66:75-78.

Tanaka, K. 1976. Oviposition-including activity in the ovarian follicles of different sizes in the laying hen. Poult. Sci. 55:714-716.

Tanaka, K. and T. Goto. 1976. Partial purification of the oviposition-inducing factor and extimation of its chemical nature. Poult. Sci. 55:1774-1778.

Tanaka, K., K. Goto, T. Yoshioka, T. Terao and O. Koga. 1984. Changes in the plasma concentration of immunoreactive arginine vasotocin during oviposition in the domestic fowl. Brit. Poul. Sci. 25:589-595.

Tennyson, V. M., A. Hou-Yu, G. Nilaver and E. A. Zimmerman. 1985. Immunocytochemical studies of vasotocin and mesotocin in the hypothalamo-hypophysial system of the chicken. Cell Tissue Res. 239:279-291.

Wathes, D. C., R. W. Swann, M. G. R. Hull, J. O. Drife, D. G. Porter and B. T. Pickeing. 1983. Gonadal sources of the posterior pituitary hormones. Prog. Brain Res. 60:513-520.

Wathes, D. C., R. W. Swann, B. T. Pickeing, D. G. Porter, M. G. R. Hull and J. O. Drife. 1982. Neurohypophysial hormone in the human ovary. Lancet II:410-412.

Watkin, W. B. and V. J. Choy. 1988. Identification of neurohypophysial peptides in the ovaries of several mammalian and nonmammalian species. Peptides. 9:927-932.

Yoshimura, Y., K. Tanaka and O. Koga. 1983. Studies on the contractility of follicular wall with special reference to the mechanism of ovulation in hens. Brit. Poul. Sci. 24:213-218.