Mesotocin Increases the Sensitivity of the Hen Oviduct Uterus to Arginine Vasotocin

T. Takahashi and M. Kawashima[1]

Department of Avian Endocrinology, Gifu University, Yanagido, Gifu 501-1193, Japan

ABSTRACT The present study was performed to elucidate whether mesotocin (MT), one of avian neurohypophysial hormones, relates to the action of arginine vasotocin (AVT) on oviposition of hens. The ratio of AVT-induced oviposition was increased when 1 μg/hen of MT was injected together with AVT. An intravenous injection of 1 μg/hen of MT caused an increase in the binding affinity of the uterine AVT receptor and a decrease in the binding capacity. Blood MT concentrations measured by RIA increased approximately 1 min before oviposition during the period of the bearing-down behavior, but the AVT concentration did not change at this time. The blood AVT concentration dramatically increased within 1 min just after oviposition. The results suggest that MT may have an effect of enhancing the inducing oviposition by AVT through the increase in the sensitivity of the uterus to AVT at oviposition in hens.

Key words: hen, oviposition, mesotocin, arginine vasotocin action, oviduct uterus

2008 Poultry Science 87:2107–2111
doi:10.3382/ps.2008-00076

INTRODUCTION

Avian neurohypophysial hormones are mesotocin (**MT**; Acher et al., 1970) and arginine vasotocin (**AVT**; Munsick et al., 1960). Recently, it was reported that the receptor for MT exists in the oviduct uterus of hens, and its bindings (binding affinity and capacity) change relating to oviposition (Takahashi and Kawashima, 2008). It was also reported that the receptor for AVT exists in the uterus of hens (Takahashi et al., 1992), and the bindings changes during the oviposition cycle (Takahashi et al., 1994b). The concentration of the blood AVT dramatically increases just after oviposition, as is well known (Sturkie and Lin, 1966; Tanaka et al., 1984; Rice et al., 1985; Shimada et al., 1986; Takahashi et al., 1994b), and the AVT causes the contractions of the smooth muscle of the uterus (Munsick et al., 1960; Rzasa, 1972) for oviposition (Rzasa and Ewy, 1970). Although Nouwen et al. (1984) and Koike et al. (1988) measured MT concentrations in blood before and after oviposition in chickens, changes related to oviposition were not detected. The role of MT is obscure in the oviposition cycle of hens.

The egg stays in the uterus for approximately 20 h (Warren and Scott, 1935) during the eggshell formation in hens, and the egg formation is completed (Richardson, 1935). Approximately 15 min before oviposition, the hen shows a characteristic behavior (restlessness) for about 10 min (Jull, 1952). After the restlessness behavior, a bearing-down, which is another characteristic behavior, is induced by the stimulation from the vagina (Sykes, 1953; Sturkie et al., 1962), but the bearing-down is not necessarily caused by the entering of egg into the vagina (Takahashi and Kawashima, 2003). A few minutes later, the egg is transferred to the vagina and expelled outside the body by peristalsis of the vagina (Sykes, 1953). The binding affinity of the AVT receptor in the uterus increases during the restlessness behavior (Takahashi et al., 1994b). In contrast, the MT receptor binding affinity in the uterus was elucidated to increase before the increasing in the binding affinity of the AVT receptor (approximately 30 min before oviposition; Takahashi and Kawashima, 2008). However, the relation between MT and AVT in the oviposition system is obscure. The present study was performed to demonstrate the effect of MT in vivo on the AVT receptor binding in the uterus of the hen before and after oviposition.

MATERIALS AND METHODS

Birds

White Leghorn hens (20 mo of age; 1.7 to 2.2 kg of body weight) laying 5 or 6 sequential eggs with a 1-d pause between sequences for more than 2 wk were used as laying hens. Hens (20 mo of age; 1.7 to 2.1 kg of body weight) that had not laid an egg for 10 d before experiments were also used as nonlaying (molting) hens.

Received February 19, 2008.
Accepted May 2, 2008.
[1]Corresponding author: kawasima@gifu-u.ac.jp

These hens used were kept under 14 h (0500 to 1900 h) of light per day with feed (15% CP; 2,800 kcal of ME; Japan Feeding Standard for Poultry, 1992), and water was provided ad libitum. All birds were cared for and used according to the institutional guidelines of Gifu University. In the nonlaying hens used, the ovarian weight was less than 8.5 g, and whole oviduct weight was less than 14.3 g. The plasma concentrations of estradiol-17β, progesterone ($\mathbf{P_4}$), and testosterone measured by a routine RIA (Shodono et al., 1975) were less than 297 (estradiol-17β), 343 (P_4), and 284 p*M* (testosterone), respectively.

Induction of Oviposition by an Injection of AVT Without or With MT

Arginine vasotocin (Bachem AG, Bubendorf, Switzerland) and MT (Bachem AG) were dissolved in 0.05 *M* acetic acid. The AVT (0.08, 0.1, 0.15, and 0.3 μg/hen) or 0.05 *M* acetic acid (Wako Pure Chemical Industries Ltd., Osaka, Japan) vehicle (0.5 mL/hen) was injected singly or as a mixture with 1 μg of MT into the wing vein of the laying hen 16 h before oviposition of the first egg of a laying sequence. The hens were observed for 15 min after the injection, and the occurrence of oviposition was recorded. When oviposition was not observed, the existence of the egg in the oviduct was confirmed by a digital palpation through the cloaca.

Measurement of Binding Affinity and Capacity of the AVT Receptor in the Uterus After an Injection of MT

Nonlaying hens in which bindings for the uterine AVT receptor are stable during a 24-h period (Takahashi et al., 1994b) were used to examine the effect of MT on the AVT receptor. The oviduct uterus was obtained (4 birds in each group) from nonlaying hens 2, 5, and 10 min after a single intravenous injection of MT (1 μg/hen) or 0.05 *M* acetic acid vehicle (0.5 mL/hen). Preparation of the cell membrane fraction of the uterine myometrium and the method of assay for AVT receptor bindings were the same as reported earlier (Takahashi et al., 1992; Takahashi and Kawashima, 2008). Protein concentrations of the membrane fractions were measured by the method of Lowry et al. (1951) using BSA (Sekagaku Corp., Tokyo, Japan) as a standard. The specific binding of [^{125}I]AVT was expressed as picomoles per milligram of protein. The equilibrium dissociation constant ($\mathbf{K_d}$) and the maximum binding capacity were estimated by Scatchard analysis (Scatchard, 1949).

Measurement of Plasma MT and AVT Concentrations Before and After Oviposition

To collect blood samples before and after oviposition, the time before oviposition was estimated from the oviposition time observed before the experiment. The clock time of oviposition of the first egg of the laying sequence was 0654 h ± 4 min ($\bar{X}$ ± SEM, n = 50). The period of the restlessness behavior was 12′50″ ± 1′17″ ($\bar{X}$ ± SEM, n = 50) and the bearing-down behavior was 1′13″ ± 7″ (n = 50). Blood was collected 1 h before oviposition, approximately 14 min before oviposition (in the period of restlessness behavior), approximately 1 min before oviposition (in the period of bearing-down behavior), within 1 min after oviposition, and 1 h after oviposition (6 birds at each time) of the first egg of the laying sequence. Blood was also collected from the nonlaying hens at a time corresponding to the time (0600, 0700, and 1800 h) of collecting blood of the laying hens (6 birds at each time). Mesotocin and AVT were extracted from 1 mL of the plasma using an octadecasilyl-silica cartridge (Sep Pak C18, Waters Corp., Milford, MA) as reported earlier (Takahashi et al., 1994a). Eluents from the cartridge were dried by a centrifugal evaporator (CVE-100, Tokyo Rikakikai Co. Ltd., Tokyo, Japan).

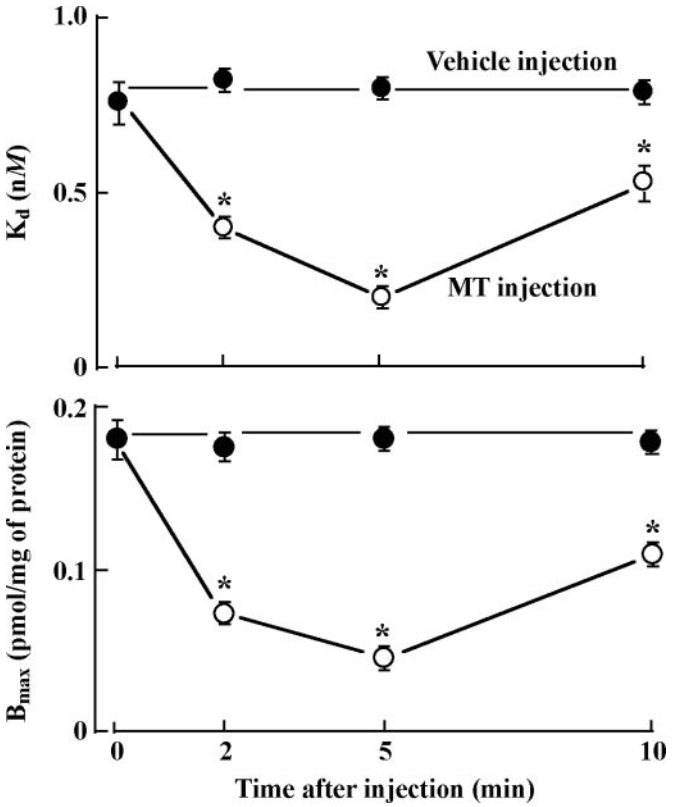

Figure 1. The equilibrium dissociation constant (K_d) and the maximum binding capacity (B_{max}) of the arginine vasotocin (AVT) receptor in the cell membrane fraction of the oviduct uterus of nonlaying hens after an intravenous injection of mesotocin (1 μg/hen: ○) or 0.05 *M* acetic acid vehicle (0.5 mL/hen: ●). Samples (10 μg of protein per tube) were incubated at 30°C for 16 h with [^{125}I]AVT (0.05 to 1.8 n*M*) in the presence and absence of 1 μ*M* unlabeled AVT, and the specific [^{125}I]AVT binding was measured. The values of K_d and B_{max} were obtained by Scatchard analysis. The amount of protein in membrane fractions, expressed as milligrams per gram of wet tissue weight, was 0.26 ± 0.02 ($_{(mean}$ ± SEM, n = 28) and was not significantly different among the groups. Each point represents the mean of 4 birds, and the vertical bars represent SEM. *Significantly different ($P \leq 0.01$) from the value of the point without asterisk by Newman-Keuls' test.

Table 1. Induction of oviposition in the hen after an intravenous injection[1] of arginine vasotocin (AVT) without or with mesotocin (MT)

	Without MT			With MT[2]		
		Oviposition[3]			Oviposition[3]	
AVT (μg/hen)	Hens injected (n)	n	%	Hens injected (n)	n	%
0 (vehicle)	20	0	0	20	0	0
0.08	20	0	0	20	8	40**
0.10	20	4	20	20	14	70**
0.15	30	19	63	30	27	90*
0.30	30	24	80	30	30	100*

[1]Sixteen hours before expected oviposition.

[2]1 μg/hen of MT was mixed with various doses of AVT.

[3]Occurrence of oviposition was observed within 15 min after the injection.

*Significantly different ($P \leq 0.05$) from the data of without MT by Fisher's exact test.

**Significantly different ($P \leq 0.01$) from the data of without MT by Fisher's exact test.

The extracts were dissolved in 200 μL of PBS (0.1 *M* phosphate, 0.15 *M* NaCl, pH 7.5) containing 0.1% BSA, and MT and AVT concentrations were measured by RIA (Goto et al., 1986) using [^{125}I]MT and [^{125}I]AVT labeled by a method (Takahashi et al., 1993) using Iodogen (Sigma Chemical Co., St. Louis, MO). The sensitivity of the assay as reflected by the dose at which 90% of labeled MT and AVT was bound was 0.5 ± 0.05 p*M* ($\bar{X}$ ± SEM, n = 5; MT) and 1.2 ± 0.17 p*M* (n = 5; AVT), and the dose at which 50% of labeled MT and AVT was bound was 6.4 ± 0.75 p*M* (n = 5; MT) and 9.7 ± 0.87 p*M* (n = 5; AVT), respectively. Antisera of the MT and AVT (R2-Kyushu) used were kindly provided by Kiyoshi Shimada of Nagoya University and by Kousaku Tanaka of Kyushu University, respectively. The recovery ratio of labeled hormones from the plasma was 80.8 ± 2.4% (n = 6). The intra- and interassay coefficients of variation were 6.8 and 7.3% in MT assay and 8.1 and 12.8% in AVT assay, respectively.

Statistical Analyses

The data were analyzed by 1-way ANOVA (Snedecor and Cochran, 1967). When significant ($P \leq 0.05$) effects were found, Newman-Keuls' multiple range test (Snedecor and Cochran, 1967) was used to compare means of more than 2 groups. The Fisher exact test for 2 × 2 tables was used for analyzing differences in the percentages of the occurrence of oviposition.

RESULTS

Effect of MT on AVT-Induced Oviposition and the Binding Affinity and Capacity of AVT Receptor in the Uterus

The ratio of oviposition induced by an injection of AVT with 1 μg/hen of MT was greater than by an injection of AVT without MT (Table 1). Values of K_d and maximum binding capacity did not change in vehicle-injected hens but decreased in MT-injected hens 2, 5, and 10 min after the injection (Figure 1).

MT and AVT Concentrations in the Plasma Before and After Oviposition

The plasma MT concentration of laying hens increased just before oviposition (approximately 1 min before oviposition) during the period of bearing-down behavior and decreased just after oviposition (within 1 min). The AVT concentration of plasma in laying hens did not show apparent changes before oviposition but increased dramatically just after oviposition (Table 2). As for nonlaying hens, neither the MT nor the AVT concentration in plasma changed at a time corresponding to the time of collecting blood of laying hens (Table 2).

DISCUSSION

An injection of 1 μg/hen of MT did not induce oviposition, but the oviposition ratio after an AVT injection was increased when MT was injected together with AVT (Table 1). This result suggests that MT may have an effect to enhance the inducing oviposition by AVT. The effect of the enhancement by MT on AVT-induced oviposition may be carried by an increase in the binding affinity of the AVT receptor in the uterus, because an injection of 1 μg/hen of MT decreased the K_d value (i.e., increased the binding affinity) in the AVT receptor of the uterus (Figure 1). The binding capacity of the uterine AVT receptor also decreased after a MT injection. Although the exact reason for the decrease in the binding capacity is obscure, it might be the result of an increased binding of AVT to the receptor in vivo due to the increase in the binding affinity. It has also been reported that a decrease in the binding affinity of the AVT receptor is followed by a decrease in the binding capacity after administrations of ovarian steroid hormones (Takahashi et al., 1992).

Table 2. Plasma concentrations of mesotocin (MT) and arginine vasotocin (AVT) in laying hens at various times before (−) and after (+) oviposition and of nonlaying hens at corresponding times

Time before (−) and after (+) oviposition[1]	Clock time (h)	MT (p*M*)		AVT (p*M*)	
		Laying hen	Nonlaying hen	Laying hen	Nonlaying hen
−1 h	0600	8.73 ± 1.19[A,2]	10.77 ± 0.61[A]	7.45 ± 0.55[A]	7.40 ± 0.20[A]
Approximately −14 min of (restlessness)[3]		7.82 ± 1.60[A]		9.05 ± 3.75[A]	
Approximately −1 min of (bearing-down)[4]	0700	45.46 ± 7.41[B]	9.90 ± 1.60[A]	11.75 ± 3.14[A]	7.40 ± 1.20[A]
Within +1 min		8.32 ± 1.30[A]		90.83 ± 12.75[B]	
+1 h	0800	8.34 ± 0.56[A]	9.75 ± 0.59[A]	9.20 ± 0.53[A]	6.53 ± 0.41[A]

[A,B]Means in the same column with no common superscript differ significantly by Newman-Keuls' multiple range test ($P \leq 0.01$).

[1]Spontaneous oviposition of the first egg of the laying sequence.

[2]Values are $\bar{X}$ ± SEM of 6 birds.

[3]Restlessness behavior was 12′50″ ± 1′17″ ($\bar{X}$ ± SEM, n = 50), and blood was collected during the restlessness behavior.

[4]Bearing-down behavior was 1′13″ ± 7″ ($\bar{X}$ ± SEM, n = 50), and blood was collected during the bearing-down behavior.

Recently, it was reported that the binding affinity and capacity of the MT receptor change in relation to oviposition in hens (Takahashi and Kawashima, 2008). The binding affinity of the uterine MT receptor increases 30 min before oviposition (Takahashi and Kawashima, 2008). The binding affinity of the AVT receptor in the uterus also increases at a close time of oviposition (Takahashi et al., 1994b), but that time is different from the time of increasing in the MT receptor (Takahashi and Kawashima, 2008). The increase in the binding affinity of the AVT receptor occurs during the restlessness behavior (i.e., approximately 13 min before oviposition in this experiment). The cause of the increase in the binding affinity of the uterine AVT receptor may be due to the effect of P_4 as reported earlier (Takahashi et al., 1994b). But, it may be not only by P_4 but also by MT, because the binding affinity of the MT receptor in the uterus increased earlier than the increase in that of AVT receptor (Takahashi and Kawashima, 2008), and the binding affinity of the AVT receptor in the uterus was increased by MT (Figure 1).

The MT and AVT concentrations in blood plasma of laying hens showed a change at an extremely near time to oviposition (Table 2). However, in nonlaying hens, both MT and AVT concentrations did not change at a time corresponding to the time of oviposition in laying hens. These results suggest that not only AVT but also MT may be released into blood relating to oviposition. The MT concentration increased approximately 1 min before oviposition, but the AVT concentration was not observed to change at this time and was dramatically greater in blood collected just after oviposition. A MT injection did not cause oviposition, but an AVT injection causes oviposition (Table 2). Because oviposition is thought to be induced by AVT, the AVT may have already been released immediately before oviposition. In addition, a time lag exists between increases in MT and AVT concentrations in laying hens. The MT may release just before the AVT release, and increased MT may enhance the action of large AVT released at oviposition. The present study demonstrated that MT increases the AVT action on inducing oviposition during the period before oviposition.

ACKNOWLEDGMENTS

We thank Kiyoshi Shimada and Kousaku Tanaka (Gifu University) for providing MT and AVT antibodies, respectively, and Yusuke Nozaki and Minako Takagi (Gifu University) for technical assistance.

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