【Original Article】

Specific Bindings for Arginine Vasotocin in Five Major Regions of the Oviduct of Laying Chickens (*Gallus domesticus*)

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Summary

The present study investigated whether specific binding for arginine vasotocin (AVT) is found in the oviduct infundibulum, magnum and isthmus of laying chickens and to determine the degree of binding in those tissues compared with that in the oviduct uterus (shell gland) myometrium and vagina. Using radio ligand binding assay by ^{125}I labeled AVT, specific bindings to AVT were found in the cell membrane fraction of all five major regions in the oviduct of laying chickens. Degree of specific bindings for AVT was lower in the infundibulum, magnum and isthmus than in the uterus, but not significantly different from that in the vagina. These findings suggest a possibility of the presence of AVT receptor in the oviduct infundibulum, magnum and isthmus as well as in the uterus and vagina of laying chickens.

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Key words: arginine vasotocin specific binding, laying chicken oviduct, infundibulum, isthmus, magnum

Introduction

Arginine vasotocin (AVT), one of avian neurohypophysial hormones[1)], has an effect of inducing oviposition (expulsion of an egg from the oviduct outside body) in laying chickens[2)]. The oviduct of the hen consists of five major regions, the infundibulum, the magnum, the isthmus, the uterus (shell gland) and the vagina[3)]. An egg ovulated from the ovary is received by the infundibulum. The egg passes through the magnum and the isthmus spending approximately 4 h, and then stays for some 20 h in the uterus to form the egg shell[3)]. After the eggshell formation, the egg is expelled from the uterus outside body through the vagina[4)] by the contraction of uterine smooth muscle and the peristalsis of the vagina[5)]. It is thought that the uterine contraction for oviposition is stimulated[6)] by the action of AVT, which is released from the neurohypophysis[7,8)]. *In vitro* studies have revealed that not only the uterine muscle but also the vaginal muscle[6)] is involved. Because the receptor for AVT exists in both the uterus[9)] and the vagina[10)] of laying chickens, it is suggested that AVT is related to the regulation of uterine and vaginal contractions for oviposition. Further, it has been reported that AVT also causes contractions of the muscle of oviduct magnum and isthmus *in vitro*[6)]. However the mechanism

of action of AVT in oviduct tissues is obscure excluding the uterus and the vagina. The present study investigated whether specific binding for AVT is found in the oviduct infundibulum, magnum and isthmus and, if so, to compare the degree of binding in these tissues with that in the uterus and in the vagina .

Materials and Methods

Animals and Tissues

White Leghorn laying hens (20 mo of age; 1.9 to 2.0 kg body weight) had been kept in individual cages under 14 h (0500-1900 h) light and 10 h darkness, with feed and water provided for *ad libitum*. All hens were cared and experiments were performed in accordance with the institutional guidelines of the Gifu University. Hens laying five to six sequential eggs with a l day pause between sequences were killed by decapitation just after oviposition of the first ovum of the sequence and the oviduct infundibulum, magnum, isthmus, uterus myometrium and vagina was excised.

Preparation of Cell Membrane Fractions

Cell membrane fractions of tissues of the oviduct infundibulum, magnum, isthmus, uterus myometrium and vagina were prepared under 4°C by using the same method as reported earlier[9-11]. Each tissue of the oviduct was rinsed with ice-cold Tris-EDTA buffer [TE buffer; 50 mmol/L Tris (Kishida Chemical Co., Ltd., Osaka, Japan)-HCl, 2 mmol/L EDTA (Nacalai Tesque, Inc., Kyoto, Japan), pH 7.4] containing 0.25 mol/L sucrose (Nacalai Tesque, Inc.). The tissues were weighed after blotting with a filter paper, and homogenized in the same buffer (5 vol/wt) using the Ultra-Turrax homogenizer (Type 18-10; Ika Labortechnik, Janke & Kunkel GmbH & Co KG, Staufen, Germany). The homogenate was filtered through gauze, and the filtrate was centrifuged (1,000g, 10 min). The supernatant was stored in an ice bath, and the precipitate was suspended in the same buffer. The suspension was recentrifuged and the supernatant was obtained. The supernatant was combined with the stored one and centrifuged (30,000g, 30 min). The precipitate was suspended in 5 vol/wt of the same buffer using a Potter-Elvehjem type glass-Teflon homogenizer. The suspension was gently pored on equal volume of TE buffer containing 1.0 mol/L sucrose, and centrifuged (90,000g, 90 min) in a RPS-25 swinging rotor (Hitachi Koki Co., Ltd., Hitachinaka, Japan). The interface fraction was obtained and washed twice with TE buffer not containing sucrose by centrifugations (30,000g, 30 min). The washed precipitate was suspended with a Potter-Elvehjem type glass-Teflon homogenizer, and was used as the cell membrane fraction after determinations of the protein concentration by the method of Lowry *et al*.[12] using bovine serum albumin (BSA; Seikagaku Corp., Tokyo, Japan.) as a standard.

Labeling of AVT with ^{125}I

The labeling of AVT (Bachem Inc., Torrance, CA, USA) with ^{125}I was performed as reported earlier[13] by Iodogen method[14,15]. Five micro grams of Iodogen (1, 3, 4, 6-tetrachloro-3α, 6α-diphenylglycouril; Sigma Chem. Co., St. Louis, MO, USA) dissolved in 100 μL chloroform were poured in a polypropylen tube. The tubes were used as the Iodogen-coated tube after evaporating chloroform with a stream of dry nitrogen gas. Solutions of AVT (5 μg/5 μL 0.1 mol/L acetic acid) and Na-^{125}I (1 mCi/10 μL, Amersham International plc, Buckinghamshire, UK) were mixed in the Iodogen-coated tube. The mixture was kept standing for 4 min after finger flicking for 1 min at room temperature. The reaction mixture was placed gently on a 1 cm (diameter) × 100 cm (length) Sephadex G-25 (fine type; Pharmacia LKB Biotech. AB, Uppsala, Sweden) column pretreated with 0.1 mol/L acetic acid solution containing 1% BSA (Fraction V; Sigma Chem. Co.), and eluated with

0.1 mol/L acetic acid solution containing 0.1% BSA. Eluates showing the first peak of two peaks of radioactivity after the peak of free Na-^{125}I were used for binding assays.

Binding Assay of [^{125}I]AVT to Cell Membrane Fractions

Binding assay of [^{125}I]AVT to cell membrane fractions of each tissue of oviduct was performed as reported earlier[9,10]. Polypropylene tubes (AGC Techno Glass Co., Ltd., Chiba, Japan) were pretreated overnight at 4˚C with TE buffer containing 1% BSA. Aliquots of the cell membrane fraction (10 μg protein per tube) were incubated at 30˚C for 16 h with 1 nmol/L of [^{125}I]AVT in the presence (for nonspecific bindings) or absence (for total bindings) of 1 μmol/L of unlabeled AVT in a total volume of 300 μL. After the incubation, all tubes were centrifuged (10,000g, 20 min, 4˚C), and the precipitate was washed with 500 μL of TE buffer by the same centrifugation. The radioactivity of the washed precipitate (bound ligand) was measured by an automatic gamma counter (Beckman Gamma 9000; Beckman Instruments, Fullerton, CA, USA). The counting efficiency of the counter was 71.3 to 78.0% for ^{125}I. Specific bindings were obtained by subtracting the nonspecific binding from the total binding and expressed as moles per milligram protein.

Statistical Analyses

For comparisons among more than two groups, the data were analyzed by one-way ANOVA[16]. When significant ($P \leq 0.05$) effects were found, Tukey's multiple range test[17] was used to separate means.

Results

The specific binding to AVT was found in cell membrane fractions of all of five oviduct regions (Figure 1). The degree of specific binding for AVT was not significantly different among the

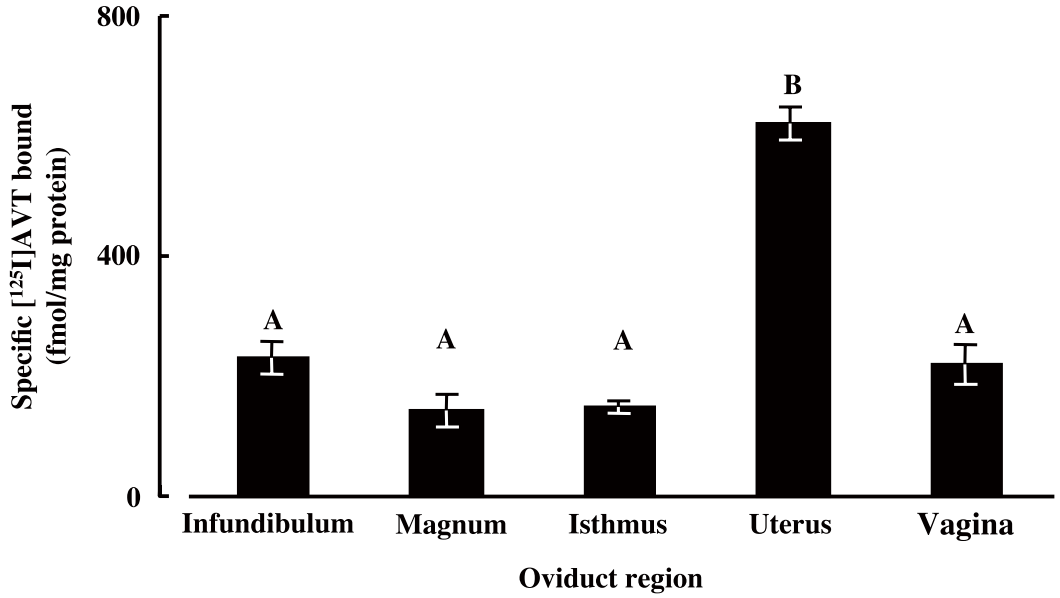

Fig. 1. Specific [^{125}I]AVT binding in the cell membrane fraction of the infundibulum, magnum, isthmus, uterus (shell gland) and vagina of oviduct in laying chickens. Cell membrane fraction samples (10 μg protein per tube) were incubated at 30℃ for 16 h with 1 nmol/L [^{125}I]AVT in the absence or presence of 1 μmol/L unlabeled AVT, and the specific [^{125}I]AVT binding was measured. Each column represents the mean of three separate pools of samples, and vertical bars represent SEM. Means with no common letter are significantly different ($P \leq 0.01$).

oviduct infundibulum, magnum and isthmus. These were lower than in the oviduct uterus, but not significantly different compared with that in the oviduct vagina.

Discussion

Specific bindings to AVT were found in the cell membrane fraction of all five major regions in the oviduct of laying chickens (Figure 1). It has been reported that receptors for AVT exist in the cell membrane fraction of the oviduct uterus myometrium[9] and vagina[10] in hens. Therefore, the specific AVT binding found in the uterus myometrium and vagina might be caused by AVT binding to those receptors. Although the presence of the AVT receptor has not been previously reported in the infundibulum, magnum and isthmus, the present findings suggest that the receptor for AVT may also exist in these oviduct tissues. AVT stimulates the contraction of smooth muscle not only in the uterus and the vagina but also the magnum and the isthmus *in vitro*[6]. This finding may also suggest a possibility of the presence of AVT receptors in the oviduct magnum and isthmus in laying hens.

The degree of specific binding for AVT did not significantly differ among the oviduct magnum, isthmus and vagina, but were lower than that in the uterus (Figure 1). This may be related to the different amounts of smooth muscle contained in these tissues. In the infundibulum, there has not been any previous report investigating whether AVT stimulates the contraction of smooth muscle, but there was an almost equal degree of specific binding for AVT compared with that in the magnum, the isthmus and the vagina. If the specific binding components in the oviduct infundibulum, magnum and isthmus are the AVT receptor, AVT may directly regulate the transport of the egg to the vagina from the infundibulum in laying chickens.

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産卵鶏の卵管の主要な5つの部位におけるバソトシンの特異的結合について

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要　旨

産卵鶏の卵管の主要な 5 つの部位、すなわち漏斗部、膨大部、峡部、子宮部（卵殻腺部）筋肉層および膣部を採取して、それぞれの部位の細胞膜画分を調製した。これらの細胞膜画分に対し、^{125}I で標識したバソトシンの結合実験を行ない、特異的結合が測定できるかどうか検討した。その結果、5 つのすべての部位においてバソトシンの特異的結合が観察され、その量は漏斗部、膨大部および峡部においては子宮部においてよりも小さかったが、膣部においてとは統計的有意差はなかった。本研究の結果から、バソトシンは産卵鶏の卵管において、子宮部と膣部においてすでに知られているように、漏斗部、膨大部および峡部においてもその受容体が存在する可能性が示唆された。

キーワード：バソトシン特異的結合、産卵鶏卵管、漏斗部、膨大部、峡部

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