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Feeding response following central administration of mesotocin and arginine-vasotocin receptor agonists in chicks (*Gallus gallus*)

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HIGHLIGHTS

- Central injections of MT and AVT induce anorexia and wing-flaps in chicks.
- The agonist for the MT receptor had no effect on these behaviors.
- The agonist for the AVT receptor induces anorexia and wing-flaps.
- Therefore the AVT but not MT receptor is related to MT- and AVT-induced behavior.

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ABSTRACT

Mesotocin (MT) and arginine-vasotocin (AVT) are posterior pituitary derived hormones in birds and are homologous to mammalian oxytocin (OT) and vasopressin (VP), respectively. We previously reported that intracerebroventricular (ICV) injection of both MT and AVT inhibit feeding and induce wing-flapping in chicks (*Gallus gallus*). Because both peptides cause similar effects suggests that they might act via common receptors. However, the specific receptors of MT and AVT which mediate their anorexigenic effect have not been clarified in chicks. Thus, the purpose of the present study was to identify the receptor subtypes involved in MT- and AVT-induced anorexia and behavioral patterns by using several agonists. ICV injection of vasopressin-1 receptor agonist (V1R) (homologous to chicken AVT receptor-2 and -4 [VT2R and VT4R, respectively]), significantly decreased food intake while agonists of vasopressin-2 receptor (V2R) and OT receptor (OTR) (homologues of chicken AVT receptor-1 and MT receptor respectively) had no effect. In addition, V1R agonist induced wing-flapping although this was not affected by V2R or OTR agonists. Since VT2R has not been found in the brain of chicks, the present study suggested that VT4R might be related to the anorexigenic effect and wing-flapping induced by MT and AVT in chicks.

1. Introduction

Mesotocin (MT), the homologue of mammalian oxytocin (OT) is a posterior pituitary hormone in birds. In mammals, several studies have demonstrated a diverse range of physiological actions associated with OT. For example, OT is related to contraction of the uterus during childbirth, facilitates lactation after stimulation of the nipple, regulates urine volume, and affects vasodilatation which lowers blood pressure [1,2,3,4]. In the mammalian central nervous system, OT is associated with changes in behavior including social interactions [5] and inhibition of feeding [6,7]. Unlike OT in mammals, the physiological significance of MT is not well clarified in birds. Recently, we reported that intracerebroventricular (ICV) but not intraperitoneal injection of MT inhibits food intake in chicks (*Gallus gallus*) [8].

Central injection of MT does not only induce anorexia but also wing-flapping in chicks [8]. Interestingly, the anorexia and wing-flapping are also observed after central injection of arginine-vasotocin (AVT) in chicks [9], which is another posterior pituitary hormone in birds and is homologous to mammalian vasopressin (VP). These findings suggest that MT and AVT might induce anorexia and wing-flapping via a common mechanism in the brain of chicks. In fact, mammalian studies revealed that interactions between OT and VP receptors are abundant [10,11,12] and OT and VP have affinity to reciprocal receptors [13,14]. Moreover, Bales et al. [15] reported that activation of either OT or VP receptors causes behavioral changes. Thus, it is possible that both MT and/

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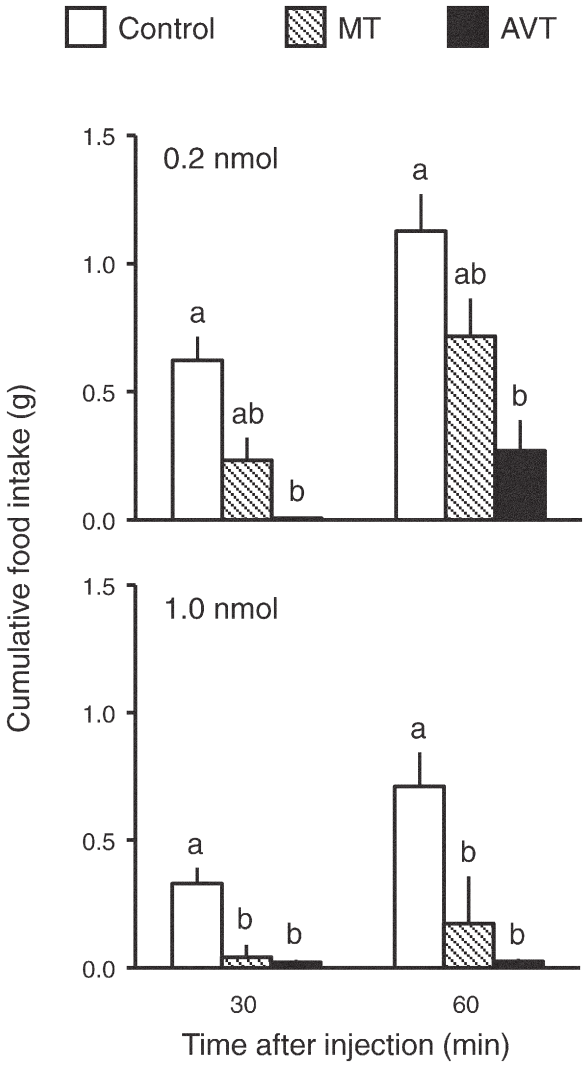

Fig. 1. Effect of ICV injection of MT and AVT on food intake in free-feeding chicks. The number of chicks in 0 (control), MT and AVT groups were 11, 11 and 9 for 0.2 nmol study, and 9, 5 and 9 in 1.0 nmol study, respectively. Data are expressed as means ± SEM. Groups with different superscripts are significantly different at each time point ($P < 0.05$).

or AVT receptors may influence behaviors of chicks. Although information about MT and AVT receptors in avian species is growing, their specific roles on behavioral changes in chicks are poorly documented.

To date, 4 types of receptors for MT and AVT have been identified in birds [16,17,18,19]. They are named as vasotocin receptor-1 (VT1R), VT2R, VT3R and VT4R. Among them, VT3R is regarded as the MT receptor (MTR) in birds [19,20] because the amino acid sequence of VT3R has the highest homology (77%) to the mammalian OT receptor (OTR). The avian VT1R, VT2R and VT4R are likely homologues to mammalian vasopressin receptor-2 (V2R), vasopressin receptor-1b (V1bR) and vasopressin receptor-1a (V1aR), respectively. Moreover, it is reported that both MT and AVT show affinity to VT1R and VT2R in vitro although the potency is different [16,17]. It is therefore possible that MT and AVT might inhibit feeding behavior in chicks via common receptor.

Conversely, the effects of MT are not completely same as those of AVT. Several studies reveal that AVT is related to antidiuretic function, oviposition and the activation of hypothalamic–pituitary–adrenal gland (HPA) axis in birds [21]. MT also regulates urine volume in hens [22] and functions as vasodepressors in cockerels [23]. However, the chicken oviduct contracts after AVT but not MT treatment [24], although MT increases the sensitivity of the hen's oviduct to AVT [25]. In chicks, moreover, ICV injection of AVT increases corticosterone (CORT) release while MT has no effect [8,9]. Thus which receptor is related to the anorexigenic effect of MT and AVT will be helpful to clarify the mechanism and the relationship between MT and AVT in chicks.

The purpose of the present study was to elucidate which receptor is related to MT- and AVT-induced anorexia and behavioral changes in chicks. This was accomplished by comparing feeding and other behavioral parameters after centrally injecting MT and AVT in chicks. Next, we investigated the effects of several agonists of MTR and VTRs on food intake and behavioral patterns.

2. Materials and methods

2.1. Animals

Day-old male layer chicks (*G. gallus*, Nihon-Layer Inc., Hyogo, Japan) were raised in a room kept at 28 °C with continuous lighting. A commercial diet and water were available ad libitum to chicks. Chicks were transferred to their individual cages 1 day before each experiment. Before the experiment, chicks were weighed and then distributed into experimental groups so that the average body weight was as uniform as possible between treatment groups. The chicks were maintained in accordance with recommendation of the National Research Council [26]. This study was approved by the Committee of Animal Care and Use in Ehime University (No. 08-o3-10).

2.2. Agonists and ICV injection

All injections were performed between 08:00 and 10:00. MT and AVT were purchased from Alpha Diagnostic International Inc., San Antonio, USA and Peptide Institute, Inc., Osaka, Japan, respectively. OTR agonist ([Thr^4, Gly^7]-OT), V1R agonist ([Phe^2, Orn^8]-OT), and V2R agonist ([deamino-cys^1, Val^4, D-Arg^8]-VP) were purchased from Bachem AG, Bubendorf, Switzerland. All peptides were dissolved in a saline solution containing 0.1% Evans Blue dye and this vehicle alone was used for the control treatment. ICV injection were performed according to a method reported previously [27]. Briefly, the head of the chicks was inserted into an acrylic box which had a hole at the top plate. The injection coordinates were 3 mm anterior to the coronal suture, 1 mm lateral from the sagittal suture, and 3 mm deep targeting the left lateral ventricle. Anatomical landmarks were determined visually and by palpation. The peptide solution was injected through the hole using a microsyringe at a volume of 10 μl. This momentary procedure does not induce stress in neonatal chicks based on food intake and CORT release [28,29]. At the end of each experiment, the chicks were euthanized with an overdose of pentobarbital. The brain was then removed to confirm the accuracy of injection. Any chicks that did not have Evans Blue dye in the lateral ventricle were not used for further analyses.

2.3. Effect of MT and AVT on food intake

Six-day-old chicks were ICV injected with vehicle (control), 0.2 nmol MT or 0.2 nmol AVT under an ad libitum feeding condition. The injected dose was decided based on previous studies using MT in chicks [8]. Then a pre-weighed feeder was given to each chick, and food intake was measured at 30 and 60 min after the injection using a digital balance at the accuracy of 1.0 mg. Similar experiments were also performed using 1.0 nmol MT and 1.0 nmol AVT.

2.4. Effect of MT and AVT on behavioral parameters

Behavioral observations were conducted for 15 min following ICV injection of MT and AVT. Five-day-old chicks were ICV injected with vehicle, 1.0 nmol MT or 1.0 nmol AVT under an ad libitum feeding condition, and then returned to their home cage. After the injection, food and water were removed from their cages. The chicks' voluntary movement