Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

Distribution of mesotocin-immunoreactive neurons in the brain of the male native Thai chicken

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ARTICLE INFO

Keywords:
Birds
Brain
Hypothalamus
Male
Mesotocin
Native Thai chicken

ABSTRACT

Mesotocin (MT), a homolog of oxytocin (OT) in mammals, is a nonapeptide neurohypophysial hormone that is mainly synthesized in specific neuronal groups within the hypothalamus and released from the posterior pituitary gland in amphibian, reptilian, and avian species. MT is associated with the neuroendocrine regulation of reproductive cycle and maternal behaviors in female native Thai chickens. Male birds exhibit parental behaviors as well. However, there are limited data regarding the role(s) of the MTergic system in males. Thus, the objective of this study was to elucidate the localization of the MT neuronal groups in the brain of male native Thai chickens. The distributions of MT-immunoreactive (-ir) neurons and fibers in the brain were studied utilizing immunohistochemistry technique. The results revealed that MT-ir neurons and fibers were distributed throughout the brain and extensively in the diencephalon. MT-ir neurons and fibers were predominantly located within the *nucleus supraopticus, pars ventralis* (SOv), *nucleus preopticus medialis* (POM), *nucleus ventrolateralis thalami* (VLT), *nucleus paraventricularis magnocellularis* (PVN), and *regio lateralis hypothalami* (LHy), suggesting that MT neurons in these nuclei might be involved in the reproductive activities and/or parental behavior in the male chickens. In addition, the numbers of MT-ir neurons within the SOv and POM were significantly higher than those of the VLT, PVN, and LHy. More importantly, the number of MT-ir neurons in the SOv was high in the male brain when compared with the female brain, indicating that the MTergic system in the SOv might play a significant role in male reproductive activities in this equatorial species.

1. Introduction

Mesotocin (MT), a homolog of oxytocin (OT) in mammals, is a nonapeptide neurohypophysial hormone that is synthesized in specific neuronal groups within the hypothalamus and released from the posterior pituitary gland into the hypophysial portal blood via the *eminentia mediana* (median eminence; ME) in amphibian, reptilian, and avian species (Bentley, 1997). MT in birds and OT in mammals are different at amino acid position 8; MT is isoleucine, but OT is leucine (Acher et al., 1970; Parry et al., 2000). MT is also distributed within several regions of the avian brain (Chokchaloemwong et al., 2013; Robinzon et al., 1988a; Thayananuphat et al., 2011) and several tissues of the reproductive tract including the corpus luteum, follicle, uterus, and placenta (Parry et al., 2000; Robinzon et al., 1988b). To date, there are a limited number of studies regarding the role(s) of MT in birds. It has been reported that MT facilitates uterine contractions in hens (Takahashi and Kawashima, 2008a, 2008b; Takahashi et al., 1997), acts as a vasodepressor in cockerels (Robinzon et al., 1994), inhibits feeding behavior in chicks (Masunari et al., 2016, 2013), and promotes sociality of female zebra finches and field sparrows (Goodson et al., 2009). The role of MT in avian brooding behavior was first documented in turkeys (Thayananuphat et al., 2011). Moreover, it has been reported that MT is involved in the regulation of the reproductive cycle and rearing behavior in the female native Thai chickens (Chokchaloemwong et al.,

Abbreviations: AM, *nucleus anterior medialis hypothalami*; BST, *bed nucleus of the stria terminalis*; Cb, cerebellum; DA, dopamine; DLAmc, *nucleus dorsolateralis anterior thalami, pars magnocellularis*; GCt, *substantia grisea centralis*; HL, *nucleus habenularis lateralis*; IH, *nucleus inferioris hypothalami*; IN, *nucleus infundibuli hypothalami*; -ir, -immunoreactive; LHy, *regio lateralis hypothalami*; ME, *eminentia mediana* (median eminence); MPOA, *area praeoptica medialis*; MT, mesotocin; NR, non-rearing hens; OM, *tractus occipitomesencephalicus*; OT, oxytocin; OVLT, *organum vasculosum lamina terminalis*; PHN, *nucleus periventricularis hypothalami*; POA, *area praeoptica*; POM, *nucleus preopticus medialis*; POP, *nucleus preopticus periventricularis*; PRL, prolactin; PVN, *nucleus paraventricularis magnocellularis*; PVO, *organum paraventriculare*; R, rearing hens; ROT, *nucleus rotundus*; SCNm, *nucleus suprachiasmaticus, pars medialis*; SL, *nucleus septalis lateralis*; SON, *nucleus supraopticus*; SOv, *nucleus supraopticus, pars ventralis*; SSO, *organum subseptale* (supseptal organ); TSM, *tractus septomesencephalicus*; V III, *ventriculus tertius* (third ventricle); VIP, vasoactive intestinal peptide; VLT, *nucleus ventrolateralis thalami*

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http://dx.doi.org/10.1016/j.acthis.2017.10.004
Received 14 July 2017; Received in revised form 13 October 2017; Accepted 16 October 2017

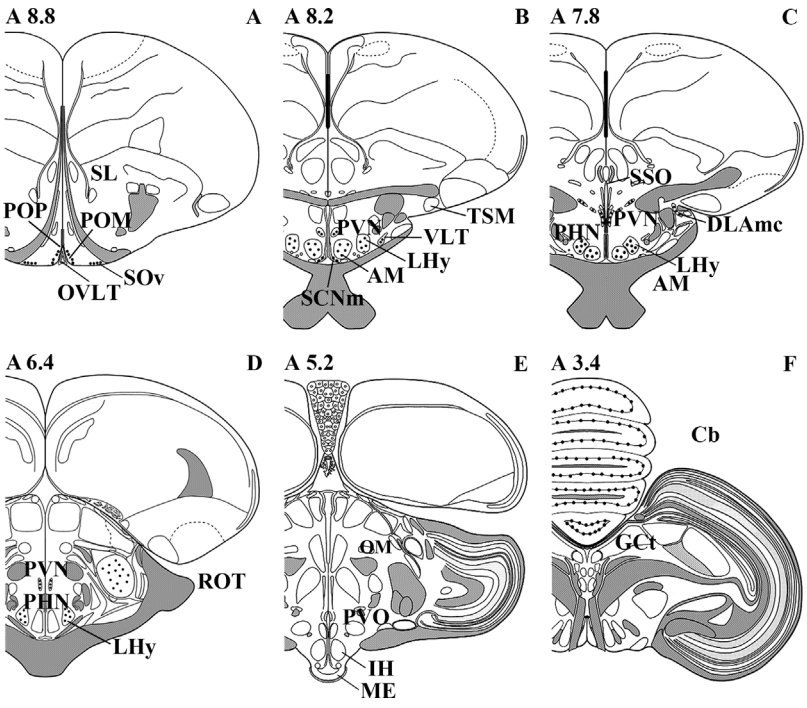

Fig. 1. Schematic diagrams of coronal sections illustrating the areas of the chick brain showing the distributions of MT-ir neurons (black dots) and fibers throughout the brain of the male native Thai chicken. Coronal illustrations were redrawn from the stereotaxic atlas of the chick brain (Kuenzel and Masson, 1988). The following abbreviations are used in the figure legends: AM, *nucleus anterior medialis hypothalami*; Cb, cerebellum; DLAmc, *nucleus dorsolateralis anterior thalami, pars lateralis*; GCt, *substantia grisea centrlis*; IH, *nucleus inferioris hypothalami*; LHy, *regio lateralis hypothalami* (lateral hypothalamic area); ME, *eminentia mediana* (median eminence); OM, *tractus occipitomesencephalicus*; OVLT, *organum vaculosum lamina teminalis*; PHN, *nucleus periventricularis hypothalami*; POM, *nucleus preopticus medialis*; POP, *nucleus preopticus periventricularis*; PVN, *nucleus paraventricularis magnocellularis*; PVO, *organum paraventriculare*; ROT, *nucleus rotundus*; SCNm, *nucleus suprachiasmaticus, pars medialis*; SL, *nucleus septalis lateralis*; SOv, *nucleus supraopticus, pars ventralis*; SSO, *organum subseptale* (supseptal organ); TSM, *tractus septomesencephalicus*; VLT, *nucleus ventrolateralis thalami*.

2013).

In birds, numerous studies have been reported that MT neurons and fibers are distributed in several regions of the brain such as the *nucleus supraopticus, pars ventralis* (SOv); ventral part of the *nucleus supraopticus* (SON), *tractus septomesencephalicus* (TSM), *nucleus preopticus periventricularis* (POP), *bed nucleus of the stria terminalis* (BST), *nucleus paraventricularis magnocellularis* (PVN), *nucleus habenularis lateralis* (HL), *nucleus inferioris hypothalami* (IH), cerebellum (Cb), lateral septum, optic lobe, tuberomammillary nucleus, pons, and medulla oblongata (Barth et al., 1997; Bons, 1980; Goossens et al., 1977; Robinzon et al., 1988a; Thayananuphat et al., 2011). MT fibers are extensively distributed within internal and external layers of the ME (Bons, 1980; Goossens et al., 1977). Similarly to the previous findings, in female native Thai chickens, the highest accumulations of MT-immunoreactive (-ir) neurons and fibers are concentrated within the SOv, *nucleus preopticus medialis* (POM), *nucleus ventrolateralis thalami* (VLT), *regio lateralis hypothalami* (LHy), and PVN. Changes in the numbers of MT-ir neurons within the SOv, POM, and PVN are associated with the reproductive stages of the native Thai chickens, with the highest density observed in the incubating and rearing hens. In addition, the numbers of MT-ir neurons within the SOv, POM, and PVN of rearing hens (R) are higher than those of non-rearing (NR) hens in these nuclei. These findings indicate that MT-ir neurons play a regulatory role in reproductive activities and the neuroendocrine reorganization to establish and maintain rearing behavior in this species (Chokchaloemwong et al., 2013).

Native Thai chicken (*Gallus domesticus*), an equatorial, tropical, non-seasonally breeding species, is originated from the wild jungle fowl in Southeast Asia. It has been domesticated without genetic selection. It expresses strong maternal behaviors which are inherited from the ancestor, the wild jungle fowl (Austic and Nesheim, 1990; Fumihito et al., 1994; Hillel et al., 2003; Sawai et al., 2010). It is well established that the neuroendocrine regulation of reproductive cycle and maternal behaviors in the female native Thai chickens is associated with gonadotropin releasing hormone, vasoactive intestinal peptide (VIP), dopamine (DA), prolactin (PRL), and MT (Chaiyachet et al., 2013a, 2013b; Chokchaloemwong et al., 2015, 2013; Prakobsaeng et al., 2011; Sartsoongnoen et al., 2012). As aforementioned, the neuroendocrine regulation of reproductive behaviors has been extensively studied, particularly in females. However, there are limited data regarding the neuroendocrine regulation of reproductive activities in males. Recently, it has been reported that VIP neurons within the IH and *nucleus infundibuli hypothalami* (IN) and its relationship to PRL and testosterone play a pivotal role in its year-round reproductive activity in the male native Thai chickens (Kamkrathok et al., 2016). In addition, male birds exhibit parental behaviors such as nest building, brooding, and feeding of the young in many species (Chaiseha and El Halawani, 2015; Lynn et al., 2015). To date, there has been no report of the MTergic system in the male native Thai chicken. Thus, the objective of this study was to investigate the localization of the MT neuronal groups in the brain of the male native Thai chickens, enabling further studies of neuroendocrinology related to behavior. The findings of the distributions of MT-ir neurons and fibers might be related to the regulation of reproductive activities and paternal behaviors in the male native Thai chickens.

2. Materials and methods

2.1. Experimental animals

Male native Thai chickens, 12 months of age, were used. They were reared and housed together with mature females (1 male: 8 females) in floor pens equipped with basket nests under natural light (approximately 12 h of light and 12 h of darkness; 12L: 12D) with free access to feed and water. The animal protocols used adhered to the guidelines approved by the Suranaree University of Technology Animal Care and Use Committee.

2.2. Experimental design

To determine the distributions of MT-ir neurons and fibers in the brain of the male native Thai chicken, 6 mature males (12 months of age) were used. The brains of mature roosters were fixed by pressure perfusion with 4% paraformaldehyde (#416780010, Acros Organics, Inc., New Jersey, USA). Tissue sectioning was performed in the coronal plane at a thickness of 16 μm utilizing a cryostat and further processing by immunohistochemistry according to a previously described method (Chokchaloemwong et al., 2013). In this study, the primary and secondary antibodies used for detecting MT immunoreactivity were