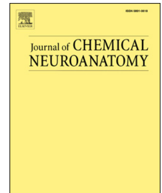
Journal of Chemical Neuroanatomy 94 (2018) 1–7

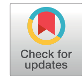
Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

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



Reproductive status affects the expression of prolactin receptor mRNA in the brain of female Damaraland mole-rats



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ARTICLE INFO

Keywords:
Prolactin receptor
Social status
In situ hybridization
Fukomys damarensis
Prolactin

ABSTRACT

The eusocial Damaraland mole-rat (*Fukomys damarensis*) represents an extreme example of reproductive skew, in that reproduction is completely blocked in female subordinate group members. It is thought that in these animals normal GnRH secretion from the hypothalamus is disrupted. Prolactin, a peptide hormone secreted from the anterior pituitary gland, has been implicated in a wide variety of functions. Well documented in rodents is its role in mediating lactational infertility. Elevated circulating prolactin levels, such as during lactation, are associated with reduced GnRH release into the portal blood and with a reduction in the frequency and amplitude of LH pulses. The present study aimed at investigating whether such a mechanism could act in reproductively suppressed female Damaraland mole-rats. By means of *in situ* hybridisation we studied the distribution and gene expression of the prolactin receptor (*Prlr*) in wild-caught female Damaraland mole-rats with different reproductive status. Substantial *Prlr* expression was found in several brain regions, with highest levels in the choroid plexus and moderate expression in the preoptic and tuberal hypothalamus. While in reproductive and non-reproductive females plasma prolactin levels were very low and not significantly different, quantification of the *Prlr* hybridisation signal revealed significant differences in relation to reproductive status. Reproductively suppressed females had increased expression of *Prlr* in the choroid plexus and in the arcuate nucleus (ARC) when compared to reproductive females. This suggests higher local prolactin levels in the brain of suppressed females. Together with previous findings, it could indicate that prolactin inhibits ARC kisspeptin neurons, which then would lead to reduced activation of GnRH neurons in such females.

1. Introduction

In mammals, prolactin receptors have been found in a wide variety of tissues and consequently, numerous biological functions of prolactin have been described (Kelly et al., 1991; Bole-Feysot et al., 1998; Freeman et al., 2000). The neuroendocrine functions of prolactin include the stimulation of maternal behaviour, suppression of fertility, suppression of stress response, regulation of food intake and body mass and activation of hypothalamic dopaminergic neurons to regulate its own secretion (Grattan and Kokay, 2008). Well documented in humans and rodents is the suppression of reproduction during lactation, where high circulating levels of prolactin are associated with an inhibition of gonadotropin secretion and leading to anoestrus or amenorrhoea. Similar effects are also observed in hyperprolactinemic non-lactating females (McNeilly, 1980, 2001).

In group-living mammals, reproductive suppression, where dominant individuals inhibit the reproductive capabilities of subordinates *via* behavioural and other interactions, is commonly found (Keller and Reeve, 1994). Extremes of high reproductive skew are represented by societies with a single breeding pair and helpers, such as in callitrichid primates (Abbott, 1984), mongooses and meerkats (Rasa, 1973; Doolan and Macdonald, 1997) and African mole-rats (Bennett and Faulkes, 2000). The mole-rats of the family Bathyergidae exhibit a wide range of social behaviour and restricted reproduction has been found in several species (Bennett and Faulkes, 2000). Highly social Damaraland mole-rats (*Fukomys damarensis*) represent an extreme example of reproductive skew, in that reproduction is completely blocked in female subordinate group members (Bennett, 1994; Bennett et al., 1994). This blockade results from an inhibition of ovulation, which is caused by a disruption in the normal GnRH secretion from the hypothalamus (Molteno et al., 2004). Despite the potential for prolactin in being involved in the mechanism of reproductive suppression in highly social African mole-rats, its possible role remains elusive.

In rats and mice, elevated circulating levels of prolactin (hyperprolactinemia) are associated with a reduction in the frequency and amplitude of LH pulses and a suppression of GnRH release into the

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https://doi.org/10.1016/j.jchemneu.2018.08.002
Received 16 May 2018; Received in revised form 12 July 2018; Accepted 13 August 2018
Available online 15 August 2018
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portal blood (Koike et al., 1984; Cohen-Becker et al., 1986; Fox et al., 1987; Grattan et al., 2007). The reduced activity of the GnRH neurons is considered the principal cause of infertility. This suppressive effect of prolactin is most likely mediated indirectly through prolactin-sensitive afferents such as kisspeptin because only a small percentage of GnRH neurons express the prolactin receptor (Prlr, Grattan et al., 2007; Kokay et al., 2011). Prolactin secretion is under the control of dopamine, which is produced in several distinct hypothalamic dopaminergic neuron populations that express prolactin receptor. Increased prolactin levels lead to the activation of the tuberoinfundibular dopaminergic neurons (TIDA) and to increased dopamine synthesis, which after transport to the anterior pituitary binds to type-2 dopamine receptors and inhibits prolactin secretion. During lactation, the state of physiological hyperprolactinemia, this feedback mechanism is not functional and the elevated prolactin level does not activate TIDA neurons (Grattan and Kokay, 2008).

Kisspeptin, a product of the *Kiss1* gene, is a strong activator of GnRH neurons and is considered essential for reproduction and the timing of puberty onset in mammals. *Kiss1*-expressing neuron populations have been found mainly in two regions of the hypothalamus, the anteroventral periventricular nucleus (AVPV) and in the arcuate nucleus (ARC, Smith et al., 2006). *Prlr* is expressed in both *Kiss1* neuron populations (Kokay et al., 2011). In lactating rats, when prolactin levels are elevated, there is a pronounced upregulation of Prlr protein and downregulation of *Kiss1* mRNA and protein expression in the ARC (Pi and Grattan, 1999a; Yamada et al., 2007; Araujo-Lopes et al., 2014). Moreover, chronic infusion of prolactin in female mice has been found to suppress oestrus cyclicity, GnRH and LH release and to reduce hypothalamic *Kiss1* mRNA expression (Sonigo et al., 2012). These data indicate that the prolactin-induced suppression of GnRH release is mediated by inhibition of *Kiss1* neurons.

In Damaraland mole-rats we have previously shown that reproductive status affects the neuroendocrine phenotype of females with reproductively suppressed females having reduced gene expression of steroid hormone receptors in several preoptic-hypothalamic brain regions, including the AVPV and the ARC. Moreover, such females also exhibit reduced *Kiss1* expression in the ARC compared to reproductively active females (Voigt et al., 2014; Voigt and Bennett, 2018). In another highly social African mole-rat, the naked mole-rat (*Heterocephalus glaber*), transcriptome profiling revealed reduced expression of genes involved in dopamine metabolism in reproductively suppressed subordinate females, which remain in a pre-pubertal anovulatory state (Mulugeta et al., 2017). The present study in Damaraland mole-rats aimed at investigating whether prolactin could play a role in the mechanism of reproductive suppression of subordinate females in this species. We measured circulating prolactin levels and we used *in situ* hybridization to determine the distribution and gene expression of *Prlr* in the forebrain of reproductively active and reproductively suppressed females.

2. Materials and methods

2.1. Animals

In the current study, adult female Damaraland mole-rats (*Fukomys damarensis*) were used. Data were obtained from nine reproductives (breeder) and nine nonreproductives (nonbreeder). The females came from colonies that were captured between April and July 2015. Mole-rats were captured near the village of Black Rock, Northern Cape, South Africa (27°7´S, 22°50´E) with Hickman live-traps under permission from Northern Cape Nature Conservation authorities. Prior to sacrifice, animals were housed for a maximum period of 12 weeks in captivity under a 12L:12D cycle at 25° in plastic containers (1.0 m x 0.5 m x 0.5 m) containing wood shavings and they were fed on sweet potato, gem squash and apples. In each colony, the reproductive status was determined for all adults. Breeding females (syn. queens) could be readily distinguished from non-reproductive females by the presence of a perforate vagina and prominent teats. None of the females were pregnant or lactating at the time of sacrifice. All animals were kept in their original colonies until sacrifice. At the time of brain collection, body mass of all animals was recorded to the nearest gram. All experimental procedures were approved by the University of Pretoria Animal Ethics Committee (EC003-12).

2.2. Blood sampling and hormone assay

Upon decapitation of the animal, trunk blood was collected into heparin-coated collection tubes. Blood samples were centrifuged, the plasma collected and frozen at −40 °C until hormone assays were conducted. Plasma samples were assayed for prolactin using the commercially available enzyme-linked immunosorbent assay (Elabscience© Guinea pig prolactin ELISA kit, Catalogue No: E-EL-GP0358) according to the manufacturer’s instructions. The prolactin assay had previously been validated for *F. damarensis* (Bennett et al., 2018). The sensitivity of the assay was 0.1 ng/ml, the detection range 0.16–10 ng/ml, and coefficient of variation for repeatability was < 10%.

2.3. Brain histology

Mole-rats were killed by decapitation using a small mammal commercially available guillotine, brains were dissected out of the skull, immediately frozen on dry ice and stored at −80 °C until used. Before sectioning, brain mass was recorded to the nearest milligram. Frozen brains were cut on a cryostat into 20 µm coronal sections. The plane of the sections was adjusted to match as closely as possible the plane of the rat brain atlas (Paxinos and Watson, 2007). Sections were mounted onto Superfrost Plus slides (Menzel-Gläser, Braunschweig, Germany). *In situ* hybridization was carried out on adjacent series of sections for the localization of prolactin receptor (*Prlr*) mRNA.

2.4. Cloning of cDNA probes

Based on sequence information available from Damaraland mole-rat, PCR was used to amplify a fragment of the *Prlr* gene. Total RNA was extracted from the mole-rat hypothalamus by using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany). The synthesis of first-strand cDNA was done with SUPERSCRIPT III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany) and random primers. The resulting RNA-DNA hybrids were subsequently used in PCR to generate pieces of the appropriate gene. The forward primer was 5′−CCAGGAACCAACGGAGG ACT-3′ and the reverse primer was 5′-AGCCACTGCCCAGACCATAA-3′. PCR was carried out for 40 cycles by using the following parameters: 94 °C for 1 min, 55 °C for 45 s, 72 °C for 1 min. The amplified fragment was purified and cloned into the pCRII TOPO vector using the TOPO-TA cloning kit (Invitrogen, Karlsruhe, Germany). Resultant clones were sequenced to verify the authenticity and fidelity of the amplification. The cloned *Prlr* sequence [GenBank: MF944110] is 624bp in length and shows 78% homology with mouse prolactin receptor [GenBank: NM_011169.5]. Different isoforms of the prolactin receptor have been described in several species including rat and mouse, which differ mainly in the composition of the cytoplasmic domain (Bole-Feysot et al., 1998). Our *Prlr* sequence covers the extracellular and transmembrane domains (exons 4–8), which are common to short and long forms. Therefore, both isoforms are detected with our probe.

2.5. In situ hybridization

The expression of prolactin receptor in brain sections was detected with antisense RNA probes labelled with ^{35}S-CTP as described before (Voigt et al., 2014). Labeling of the probes with ^{35}S-CTP (1250 Ci/mmol; Perkin Elmer, Rodgau, Germany) was performed using the Riboprobe System (Promega). Our *in situ* hybridization procedure

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