Nucleolus-like bodies in the pineal gland of the adult yak (*Bos grunniens*)

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The pineal glands of adult yak were studied electron microscopically. Nucleolus-like bodies (NLBs) were found mostly in the pinealocytes and the interstitial cells of the pineal glands of the yak. The NLBs were electron-dense, round or ovoid bodies with a diameter of 50 nm – 500 nm. Two types of granules were identified as melanin. These may correspond to different stages of a progressive storage of melanin. Rough endoplasmic reticula with abundant ribosomes were observed. There was no correlation between the number of NLBs and the sex of the animals.

Introduction

The yak (*Bos grunniens*) is an important domestic animal on the Qinghai-Tibetan Plateau, where they number more than 13 million, constituting about 90% of the world yak population. Yaks are an important resource for farm products such as meat, milk, fibre and hides and they are also used as beasts of burden.

In most vertebrates, the pineal body is located on the roof of the diencephalon. It has nervous and endocrine properties (Jin *et al*. 2003). It secretes mainly melatonin at night and plays an important role in regulation of the seasonal reproductive function of animals and affects the age of sexual maturity in mammals (Lerner, Case & Takahashi 1960; Luboshitzky & Lavie 1999). Inclusion bodies in pinealocytes of the mammalian pineal gland have been described in man, as in other mammals, in previous studies (Calvo *et al*. 1988; Cozzi & Ferrandi 1984; Koshy & Vettivel 2001; Meyer-Arendt & Santamarina 1956). These inclusion bodies may represent nucleolus-like bodies (NLBs), mineral deposits, or secretory products (Karasek *et al*. 1983), as well as cytoplasmic inclusion bodies (CIBs) and two kinds of nuclear inclusions, coiled bodies and granular inclusion bodies. These bodies, components of the germinal vesicles (Inoue *et al.* 2011), are not usually bounded by a membrane (Matsushima *et al*. 1984). They are defined as NLBs (Fechner 1986; Kleshchinov 1989; Takeuchi 1980). The emergence of NLBs has been studied in the neuronal cytoplasm of the mouse arcuate nucleus and hypothalamus (Anzil, Herrlinger & Blinzinger 1973; Kleshchinov 1989; Santolaya 1973). The NLBs in neurons of rat sympathetic ganglia were studied by means of ultrastructural cytochemistry (Santolaya 1973; Takeuchi & Takeuchi 1982; Zareba-Kowalska, Cidadão & Daxid-Ferreira 1990). Similar structures were found in neurons of the tipulid spermatocytes by Fuge (1976) and early post-implantation embryos of rats by Takeuchi (1980).

NLBs in the pineal gland of the yak have not previously been studied. The purpose of this study is to describe the ultrastructural features of the NLBs in the pineal gland of the yak.

Materials and methods

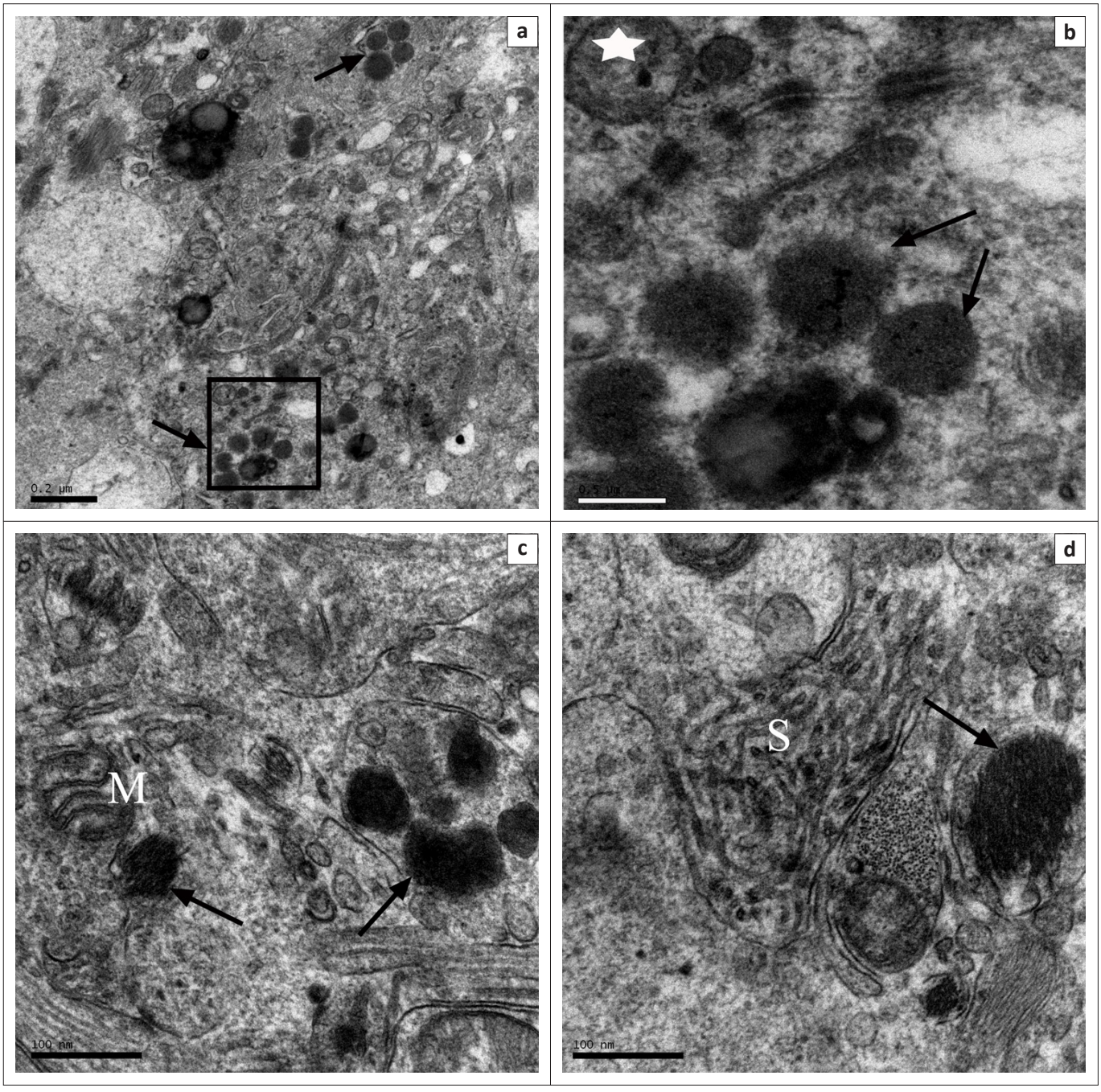
Samples from 5 animals between 6 and 10 years of age were collected in September 2011 and November 2011. The three males and two females were sexually active and were kept under natural conditions. The yaks were killed by exsanguination in the Second Yak Slaughterhouse, Datong, Qinghai, China.

After removing the calvarias, the brain was dissected and the whole pineal gland was removed. The outer margins of the pineal gland were carefully cut into tissue blocks of about 1 mm^3 and fixed in 2.5% glutaraldehyde prepared in phosphate buffer (PB, pH 7.35). Slices from representative parts of the pineal gland were postfixed in 1% osmium tetroxide prepared in PB. After a thorough wash in 5% sucrose in 0.1 m PB, the tissue slices were dehydrated in a graded series of ethanol solutions and cleared in propylene oxide. Infiltration was carried out in Epon 812. Semi-thin sections (1 µm thickness), at least three from each tissue block at different levels, were obtained using an ultramicrotome (Leica, Germany) and stained in toluidine blue O (TBO). The slides were observed with a photomicroscope (Nikon TE2000-U, Japan). Ultrathin sections (70 nm – 80 nm) of the resin blocks were obtained using the ultramicrotome. The sections were loaded on copper grids and stained with 1% ethanolic uranyl acetate followed by lead citrate.

An electron microscope (Hitachi H-7100, Japan) was used to examine the ultrathin stained tissue sections.

Results

The ultrastructure of the NLBs was similar in appearance irrespective of the area from which the samples were taken. At electron microscopic level, these bodies were found mostly in the pinealocytes and interstitial cells of the pineal gland (Figure 1a and Figure 1c). Stained with uranyl acetate and lead citrate, they were similar in appearance to the nucleoli. The inclusion bodies were spherical or ovoid and measured 50 nm – 500 nm in diameter; similar to lysosomal dense bodies, they had a dark central area consisting of unbounded granular material. They displayed a uniform texture with a few small lucent areas whose electron transparency resembled that of the cytoplasm (Figure 1a). Another type of inclusion body had electron-dense granules surrounded by a thin membrane. These were haphazardly arranged in the cytoplasm without any particular relationship or contiguity (Figure 1a and Figure1d). The dense bodies were identified as lysosomes, and microtubules were seen in the cytoplasm (Figure 1b). Mitochondria and Golgi complexes were found



a, bar = 0.2 µm; b, the larger NLBs consist of electron-dense granules without surrounding membrane (arrow), bar = 0.5 µm, hollow star, lysosomes; c, bar = 100 nm; d, inclusion bodies with electron-dense granuless are surrounded by a thin membrane (arrow), bar = 100 nm.
M, mitochondria; S; Smooth endoplasmic reticulum.

FIGURE 1: Nucleolus-like bodies in the pineal gland of adult yaks (a) an ultrathin section illustrating several NLBs in the parenchyma cells, (b) Higher magnification of the area delineated in the larger NLBs consist of electron-dense granules without surrounding membrane, (c) several NLBs in the pinealocytes (arrows) and (d) NLBs in the terminal of the dendrite.

in other pinealocytes (Figure 1c). The inclusion bodies were also observed in the terminal of the dendrite in the median plane of the superficial part of the pineal gland. Rough endoplasmic reticula with abundant ribosomes were clearly evident (Figure 1d). There was no correlation between the number of NLBs and the sex of the animals.

Discussion

Large numbers of inclusion bodies were clearly seen in the pineal gland of the yak using an electron microscope. Ultrastructurally, the NBLs of the yak pineal gland are similar to those described in other mammals (Calvo *et al*. 1988; Cozzi and Ferrandi 1984; Fechner 1986). Two types of granules were observed in this study and may correspond to different stages of progressive storage of melanin, as was previously found in a study of the dog pineal gland Calvo *et al*. 1988).

In previous studies, NLBs have been described as an accumulation of fine flocculent material in the pineal gland and it was suggested that this contains 5-hydroxytryptamine (5-HT) (Machado *et al*. 1968). However, Matsushima *et al*. (1984) observed that inclusion bodies in pinealocytes of the cotton rat appeared to contain protein and calcium and may represent NLBs, mineral deposits, secretory products or viral inclusions. In a previous study on dogs (Calvo *et al*. 1988), two types of granules in pinealocytes were identified ultrastructurally as melanin. These may correspond to different stages of a progressive melanin storage process. The NLBs in pinealocytes have been identified as melanin according to morphological features and histochemical properties (Calvo *et al*. 1988; Cozzi & Ferrandi 1984; Koshy & Vettivel 2001). It is concluded that the yak pineal gland is similar in ultrastructure to that of the dog pineal gland. No cellular morphologic alterations were observed during the different photoperiods of the year or reproductive state in the yak. The explanation for this structure and function requires further investigation.

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Competing interests

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Authors' contributions

Z.X. (Henan University of Urban Construction) was the project leader responsible for the experimental and project design and performed most of the experiments, P.G. (Henan University of Urban Construction) performed some of the experiments and prepared the samples. Z.X. and P.G. wrote the manuscript.

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