Tyrosine hydroxylase positive neurons and their contacts with vasoactive intestinal polypeptide-containing fibers in the hypothalamus of the diurnal murid rodent, *Arvicanthis niloticus*

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Abstract

Diurnal and nocturnal animals differ with respect to the timing of a host of behavioral and physiological events including those associated with neuroendocrine functions, but the neural bases of these differences are poorly understood. In nocturnal species, rhythms in tyrosine hydroxylase-containing (TH+) neurons in the hypothalamus appear to be responsible for rhythms in prolactin secretion. Here we investigated TH+ cells in a diurnal rodent (*Arvicanthis niloticus*, the unstriped Nile grass rat), and comparing them with those of a nocturnal rodent (*Rattus norvegicus*, Sprague–Dawley rat). We also examined relationships between TH+ cells and fibers containing vasoactive intestinal polypeptide (VIP) that are thought to originate from cells in the suprachiasmatic nucleus (SCN), the site of the primary circadian clock in mammals. The distribution of TH+ neurons was very similar in the two species except for a population of cells in the basal forebrain that was only present in grass rats. Fibers containing VIP appeared to contact neuroendocrine TH+ cells in both species. These data suggest that, though there may be subtle species differences, temporal information is likely to be carried along the same direct pathways from the SCN to the TH+ neurons in day- and night-active species.

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1. Introduction

The enzyme tyrosine hydroxylase (TH) plays a key role in the synthesis of monoamine transmitters and is found in cells in a variety of regions subserving different functions in the mammalian hypothalamus (Lookingland and Moore, 2004). The best studied populations of TH-containing (TH+) cells are those located in the arcuate (ARC; A12 neurons) and periventricular (PeVN; A14 neurons) nuclei that are directly involved in the regulation of neuroendocrine functions through their projections to the median eminence (van den Pol et al., 1984). Other populations of TH+ cells within the hypothalamus (A11, A13, A15) are thought to play more indirect roles in the regulation of endocrine systems, as well as in the modulation of forebrain structures involved in behavioral and physiological processes (Moore and Lookingland, 1995). These TH+ neurons are thought to play a role in regulating daily, or circadian, rhythms, and increasing evidence indicates that molecular processes within these cells fluctuate on a daily basis. For example, cells in the ARC undergo rhythms in PER proteins (Freeman et al., 2000; Kriegsfeld et al., 2003).

Abbreviations: 3V, third ventricle; AC, anterior commissure; ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus; BNST, bed nucleus of the stria terminalis; DAB, diaminobenzidene; DMH, dorsomedial hypothalamus; f, fornix; fr, fasciculus retroflexus; GnRH, gonadotropin releasing hormone; mt, medial terminal nucleus of accessory optic tract; opt, optic tract; OVLT, organum vasculosum of the lamina terminalis; OX, optic chiasm; PBS, phosphate buffered saline; PeVN, periventricular nucleus; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; sPVZ, subparaventricular zone; TH, tyrosine hydroxylase; THDA, tuberohypophyseal dopaminergic; TIDA, tuberoinfundibular dopaminergic; TX, triton-X; VIP, vasoactive intestinal polypeptide; ZI, zona incerta

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direct input from cells containing vasoactive intestinal polypeptide (VIP), a peptide produced within the suprachiasmatic nucleus (SCN), site of the primary circadian clock (Horvath, 1997; Blanchong and Smale, 2000; Mahoney et al., 2004). For example, the preovulatory surge in luteinizing hormone and mating behavior occur approximately 12 h apart in grass rats compared to the more traditional, nocturnal, lab rats (Rattus norvegicus) (McElhinny et al., 1997, 1999; Mahoney and Smale, 2005).

These functions are regulated by the release of gonadotropin releasing hormone (GnRH) from the hypothalamus into the pituitary from a population of hypothalamic neurons that receives direct input from the SCN and show rhythms in activity that are inverted in lab rats and grass rats (McElhinny et al., 1999; Mahoney and Smale, 2005). It is not known whether other populations of neuroendocrine cells receiving input from the SCN show rhythms that are inverted in diurnal and nocturnal species. In the current paper we develop a model with which to address this question by characterizing TH+ cells in the hypothalamus of the diurnal grass rat and evaluating the possibility that these cells receive direct input from the SCN.

2. Methods

2.1. Animals

Adult female grass rats (>60 days; n = 7 animals) bred from laboratory stock (Katona and Smale, 1997) and female Sprague–Dawley laboratory rats (SD, >90 days; Charles River, n = 4 animals) were singly housed, kept in a 12:12 light:dark cycle and provided food (Teklad rodent chow 8640, Harlan Industries) and water ad libitum. A red light (<5 lx) was left on continuously in each animal room. Grass rats were sexually mature but do not exhibit preovulatory surge in luteinizing hormone and mating behavior (McElhinny et al., 1999, 2005). It is not known whether other populations of neuroendocrine cells receiving input from the SCN show rhythms that are inverted in diurnal and nocturnal species. In the current paper we develop a model with which to address this question by characterizing TH+ cells in the hypothalamus of the diurnal grass rat and evaluating the possibility that these cells receive direct input from the SCN.

3. Results

3.1. TH+ cells and fibers

TH+ neurons were seen in a variety of clusters within the hypothalamus of both species. All the basic groups of TH+ neurons described previously in the hypothalamus of lab rats were seen here in tissue from both species. These include the A12, A13, A14 and A15 dopaminergic cells (Dahlstroem and Fuxe, 1964; Hokfelt et al., 1984) and are depicted in Figs. 1A–K and 2 for grass rats. An additional large cluster of TH+ neurons was present in the lateral hypothalamus and adjacent telencephalon of grass rats but not lab rats (Fig. 1A–F). Here we first describe this major anatomical difference we detected between the two species, followed by a description of the distribution of labeled cells that were present to the same extent in both species.

One major difference between the species was the presence in grass rats, but not lab rats, of a large cluster of TH+ neurons in a region that included portions of the hypothalamus and adjacent telencephalon (Figs. 1A–G and 3A and B). More
specifically, these areas included lateral hypothalamus, magnocellular preoptic nucleus, horizontal limb of the diagonal band of Broca and the substantia innominata. These neurons were diffusely distributed and extended across a considerable portion of the rostro-caudal axis of the hypothalamus. These were very large cells with typically three to four processes extending outward from the cell body; these processes branched extensively a very short distance from the cell body. These cells were completely absent in lab rats (Fig. 3).

In both species, in the rostral hypothalamus TH+ neurons were seen ventral to the lateral aspect of the anterior commissure (A15d cells, Figs. 1A–C and 2A). Towards the caudal end of their distribution this group of TH+ cells formed an arc extending medially to the dorsal pole of the third ventricle (Fig. 1C). These A15d cells were medium sized and most were bipolar, though some had three processes extending outward from the cell body. Some of these dendrites began to branch soon after leaving the cell body but most perikarya were not seen branching at all, or began to branch 400 μm or more from the cell body. In both species, we detected a more ventral group of TH+ cells dorsal to the lateral aspect of the optic chiasm and the supraoptic nucleus (SON; A15v cells, Fig. 1C–E). This population fanned out into the lateral hypothalamus. In grass rats, these cells were far more numerous and considerably more widespread when compared to SD rats. In both grass rats and SD rats some TH+ neurons were also present adjacent to the optic chiasm. These A15v neurons were larger than the A15d cells. Also at this level in the rostral hypothalamus, TH+ neurons were observed.
neurons were also seen more medially in the ventromedial preoptic area, in and lateral to the PeVN, and in the lateral aspect of the mid-level of the paraventricular nucleus (PVN) (A14 cells; Figs. 1A–H and 2B).

At the rostral end of their distribution A14 cells were adjacent to the organum vasculosum of the lamina terminalis (OVLT) and in the anteroventral periventricular nucleus (AVPV). These cells extended caudally into the PeVN along the third ventricle, the rostral PVN and to the retrochiasmatic area (Figs. 1C–H and 2B). These A14 cells were medium sized bi- or tri-polar cells whose processes did not appear to branch as they extended outward from the cell body. There were, however, occasional very large TH+ neurons interspersed with the smaller ones in the PeVN. There were somewhat fewer TH+ neurons in the PeVN of grass rats compared to SD rats. Some TH+ cells were present around and adjacent to the SCN in the SD rat; far fewer cells were seen in this area of grass rats. At this level there were also some very large TH+ cells between the two SCN and immediately caudal to the SCN; these were multipolar neurons that branched extensively and were more numerous in SD rats than in grass rats.

Caudally, a large and relatively tightly packed group of TH+ cells was present dorsal to and within the dorsomedial hypothalamus (DMH) and the zona incerta (ZI; A13 cells; Figs. 1H–J and 2C). These cells were all large and multipolar. Those TH+ fibers emanating from cells dorsal to the DMH extended considerable distances in a medial–lateral orientation without branching, while those emanating from cells just ventral to them extended in a variety of directions and branched more extensively.

In both species, a ventromedial cluster of small TH+ cells in the ARC became apparent just caudal to the SCN (A12 neurons; TIDA and THDA, Figs. 1H–K and 2D and E). Processes were often not seen on these cells, but those that were visible extended ventrally towards the median eminence.
Fig. 2. Photomicrographs of the A15–A11 groups of TH+ cells in the hypothalamus of grass rats depicting (A) A15 cells ventral to the anterior commissure (ac) in the rostral hypothalamus and (B) A14 cells in the rostral paraventricular nucleus (PVN). The arrow and dashed lines indicate the location of these cells (C) A13 cells dorsal to the dorsal medial hypothalamus (DMH), (D) rostral A12 cells (THDA neurons), (E) caudal A12 cells (TIDA neurons) and (F) A11 cells medial to the fasciculus retroflexus (fr). Scale bars = 100 μm.
Fig. 3. Photomicrographs of TH+ cells and fibers in the lateral preoptic area of grass rats at low power (A and B) and high power (C) and lab rats at low power (D, E). Scale bars = 100 μm in A, B, D, and E and 20 μm in C. Boxes in A and D represent the image depicted in B and E, respectively. Arrows indicate region where TH+ cell bodies are located in grass rats but not in SD rat. ox: optic chiasm.

Fig. 4. Photomicrographs depicting VIP+ fibers (blue) and TH+ cells (brown) in grass rats. (A) SCN and (B) caudal to the SCN where VIP+ fibers are most concentrated. (C) and (D) depict two examples of VIP+ fibers in apparent contact with TH+ cell bodies dorsal to the SCN. Scale bar = 20 μm.
Finally, TH+ neurons were seen between the third ventricle and the fascicularis retroflexus (fr, A11 neurons; Figs. 1K and 2F).

3.2. VIP+ cells, fibers and appositions with TH+ cells

VIP+ cells were seen within the core region of the SCN of grass rats and SD rats, as has been described previously (Smale and Boverhof, 1999; Moore et al., 2002) (Fig. 4). In both species the most dense plexus of VIP+ fibers extended dorsally and caudally from the SCN into the PeVN, the subparaventricular zone (sPVZ), and the lateral portion of the rostral PVN (Fig. 4A and B); some of these fibers continued on to the paraventricular thalamus. VIP+ fibers were also seen in some areas rostral to the SCN, including the periventricular region and the AVPV nucleus of the hypothalamus, but these were relatively sparse. A dense plexus of VIP+ fibers was also apparent in the bed nucleus of the stria terminalis (BNST). While the distribution of VIP+ fibers was the same in the two species, the fibers were generally denser in SD rats than grass rats, particularly in the lateral PVN and the sPVZ. Few VIP+ fibers were seen in the ARC of either species.

The VIP+ fiber distribution overlapped with that of TH+ cells in a pattern that was quite similar in the two species. Many VIP+ fibers were seen extending medially from the BNST to the region of the A15d TH+ cells, with which they made numerous contacts. There was also considerable overlap between VIP+ fibers and A14 TH+ cells in the lateral PVN, AVPV, much of the rostro-caudal extent of the PeVN region, and the sPVZ. In the PeVN area TH+ neurons tended to be more medial than VIP+ fibers, but there was some overlap. No VIP+ fibers were apparent in the regions of the A15v or A13 TH+ cells, or near the population of TH+ cells in either the lateral hypothalamus or in the lateral preoptic area/substantia innominata. TH+ cells were not seen in some regions where VIP+ fibers were apparent, such as in the paraventricular thalamus.

When we examined these areas of overlap between TH+ cells and VIP+ fibers at a higher magnification we saw appositions in the A14 population of cells. These contacts were most common in the sPVZ, the ventral edge of the PVN and the PeVN dorsal and caudal to the SCN. Appositions existed but were rare in A14 neurons rostral to the SCN (such as those in the AVPV). In addition, contacts were seen on TH+ cells at the dorsal edge of the SCN. Typically, all of these contacts were between a dark blue bouton-like structure on the VIP+ fiber and the TH+ cell soma, but fiber–fiber appositions were also present (Fig. 4C and D). Lastly, we detected a species difference: VIP+ fibers were present in the region of the ARC that contained TH+ cells (A12) in the SD rat but not in the grass rat.

4. Discussion

The grass rat diencephalon contains all of the fundamental populations of TH+ neurons, with similar morphologies and distributions as those seen here and previously described in lab rats (van den Pol et al., 1984), as well as in many other species (Novak and Nunez, 1998; Sanchez et al., 2000). In both SD rats and grass rats, we observed TH+ neurons in the PeVN and the PVN (A14 cells) as well as in the ARC (A12 cells); these cells are likely to play roles in the regulation of pituitary function in grass rats, as they do in SD rats. Another group of TH+ neurons, A13, was seen dorsal to and within the DMH and ZI. These neurons are less well understood, though they are known to project to the amygdala, PVN and diagonal band of broca in rats, where they are thought to influence behavior and arousal in addition to endocrine function (Lookingland and Moore, 2004).

In both SD and grass rats, TH+ neurons extended along the ventral aspect of the anterior commissure and fanned out dorsally from the optic chiasm into the lateral hypothalamus (A15 cells). In rats, these cells project to the SON, where they may play roles in the regulation of vasopressin and/or oxytocin cells (Lookingland and Moore, 2004). These TH cell groups are likely to serve similar functions in grass rats, though some aspects of their rhythmic properties are likely to be quite different. These basic populations of TH+ neurons (A11–A15) have been well described in a large range of vertebrate species, including humans (reviewed in Smeeets and Gonzalez, 2000).

Though the distributions of the classic populations of hypothalamic TH+ neurons outlined above were very similar in SD rats and grass rats, some species differences were apparent within them. For example, TH+ neurons in the lateral hypothalamus, dorsal to the optic chiasm, were more numerous and more widely dispersed in grass rats, while the opposite was true of cells adjacent to the SCN. Such differences in TH+ cell distribution raise the possibility of differences in the balance of inputs and/or projections from these cells. Populations of TH+ neurons may receive differential input such that the SCN has a greater influence on TH+ cells in SD rats than in grass rats. For example, the TIDA neurons located in the ARC project to the median eminence, there they release dopamine which then inhibits the release of prolactin from the anterior pituitary (Moore and Lookingland, 1995). In SD rats, but not grass rats, we detected VIP+ fibers in putative contact with these TH+ neurons (A12). In laboratory rats, this VIP+ pathway from the SCN to these dopaminergic neurons influences the release of prolactin secretion. In grass rats, the absence of an overlap of TH+ neurons in the lateral hypothalamus and/or the ARC with VIP fibers from the SCN suggests but this circadian signal is greatly reduced, or even absent in this diurnal species. This anatomical difference might represent a functional difference in the timing and regulation of TH+ cell activity and the resulting prolactin release and inhibition.

We detected one population of TH+ cells that was present in the brains of grass rats, but not SD rats. Specifically, grass rats had a substantial population of magnocellular multipolar TH+ neurons that extended from the lateral hypothalamus into the basal forebrain. These cells appear to represent the same population described initially in Syrian hamsters by Vincent (1988). In hamsters the TH+ neurons in this region do not contain aromatic amino acid decarboxylase (AADC), and therefore, although they can make l-Dopa, they cannot convert it into dopamine (Vincent and Hope, 1990). TH+ neurons in this general area have now been seen in the brains of Siberian hamsters (Shi and Bartness, 2000), and common marmosets,
but not appear to be present in two species of old world primates (Torres et al., 1993). Recent tract-tracing and lesion studies suggests that this region is critical for the integration of chemosensory and hormonal signals that regulate male sexual behavior in both hamsters and rats (reviewed in Swann et al., 2003). It should also be noted that some populations of TH+ cells have been described in the hypothalamus of other species that were not seen here in either grass rats or SD rats (Kitahama et al., 1998; Shi and Bartness, 2000).

Most hypothalamic TH neurons have been associated with neuroendocrine functions that are regulated by the circadian system (Lerant and Freeman, 1997; Gerhold et al., 2002; Kriegsfeld et al., 2003; Selliix and Freeman, 2003; Lookingland and Moore, 2004). VIP cells within the SCN appear to play a role in the regulation of neuroendocrine rhythms by communicating temporal information from the SCN to these cells (Moore and Lenn, 1972; Buijs et al., 2003; de la Iglesia and Schwartz, 2006). This has been shown for rhythms in both GnRH neurons that regulate the secretion of luteinizing hormone and follicle stimulating hormone (van der Beek et al., 1997b) and in populations of hypothalamic TH neurons that play important roles in the regulation of prolactin secretion from the anterior pituitary (Gerhold et al., 2001; Palm et al., 2001). The best studied of these TH cell populations are those that regulate prolactin secretion, the A14 and A12 cells, in the PeVN and ARC. A substantial body of data suggests that these neurons are rhythmic with respect to a variety of parameters, including dopamine production (Lerant and Freeman, 1997; Freeman et al., 2000).

Endocrine rhythms, apart from those of melatonin secretion, are generally inverted in diurnal and nocturnal species (Moore-Ede et al., 1983; Turek and Van Cauter, 1994), including grass rats and lab rats (McElhinny et al., 1999; Mahoney et al., 2004). Rhythms in Fos expression within GnRH neurons, as well as in the secretion of luteinizing hormone, are approximately 180° out of phase in grass rats relative to lab rats, peaking just before lights-on in the former species and just before lights-off in the latter (McElhinny et al., 1999; Mahoney et al., 2004). These cells receive direct input from the SCN, suggesting that the differences between the nocturnal and diurnal species reside somewhere within the mechanisms that couple rhythms in the GnRH cells to the clock within the SCN. The current data suggest that the same mechanisms may underlie neuroendocrine functions regulated by hypothalamic TH neurons, as they too appear to receive direct input from the SCN. Anterograde tract-tracing studies in the vervet monkey as well as lab rat have revealed SCN efferents that appear to contact TH+ cells in the AVPV, the lateral hypothalamus and the PeVN, as well as in the mediobasal hypothalamus (Horvath, 1997; Abizaid et al., 2004). In lab rats some SCN input to TH+ cells appears to contain VIP (Gerhold et al., 2001). The current data suggest that VIP fibers, likely originating in the SCN, may also project to TH+ cells in grass rats. This conclusion must be qualified by the fact that only electron microscopy can establish synapses with certainty. However, analysis at the light-level is generally supported by electron microscopy studies. For example, in lab rats contacts between VIP fibers and GnRH and TH+ cells that have been observed via light microscopy have been confirmed as synapses when tissue has been examined at the level of electron microscopy (van den Pol et al., 1984; van der Beek et al., 1997a; Gerhold et al., 2001).

If the anatomical pathways from the SCN to the TH+ neurons are indeed the same in lab rats and grass rats, as the current data suggest, this raises intriguing possibilities concerning the mechanisms that mediate differences between day- and night-active species. Specifically, it suggests that functional differences may exist along pathways extending from the SCN to TH neurons. This could occur via differences in the signals communicated along these pathways, or in the responses of TH cells to such signals. These issues can now be examined through future comparisons of functional aspects of these circuits in diurnal grass rats and nocturnal rats.

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