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Mitotic Figures in the Median Eminence of the Hypothalamus

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Abstract

The median eminence of the hypothalamus is part of the avenue by which neurosecreted hormones from the hypothalamic nuclei reach the pars nervosa (neural lobe) of the pituitary and eventually the bloodstream. Lithium treatment and osmotic stress increases the transport of neurosecretory hormones to the pituitary in the adult rat. Specialized astrocytes termed pituicytes in the pars nervosa of the pituitary participate in the secretory process and also develop considerable mitotic activity. The present work reveals similar mitotic figures in cells within the median eminence following 3 days of lithium treatment. The location and appearance of these mitoses add to the evidence that pituicytes are present in the median eminence. Moreover, mitoses occur within the ependymal (tanycyte) layer of the median eminence. Thus, the present results suggest that the tanycyte layer may contain pituicytes, indicating that the hypothalamus possesses specialized cells for modulating neurosecretion in response to osmotic challenges.

Keywords

Hypothalamic–pituitary–adrenal (HPA) axis; Lithium; Mitosis; Neural lobe of the pituitary; Pituicyte; Tanycyte

Introduction

The median eminence of the hypothalamus is located on the ventral surface of the brain, separating the third ventricle lumen from the subarachnoid space. The median eminence consists of five layers: ependymal, subependymal, fiber, reticular, and palisade layers [1, 2]. Antidiuretic hormone (vasopressin), a secretory product of the magno-cellular supraoptic and paraventricular nuclei in the hypothalamus, passes through the median eminence en

route to the pars nervosa (neural lobe) of the pituitary gland where the hormone is discharged into the bloodstream [3, 4]. In addition to the hormone-containing axons and the recipient blood vessels, the pars nervosa of the pituitary contains specialized astrocytes called pituicytes [5, 6]. The pituicytes of the pars nervosa play a role in the discharge of hormones from the axons into the bloodstream. Injection of hypertonic solutions precipitates the increased secretion of vasopressin [7]. Although pituicytes are not known to produce hormones themselves, they participate in the process of hormone secretion by altering their attachment to neurohypophyseal axons [8]. Pituicytes are closely associated with the secretion of neurosecretory granules, and proliferation of pituicytes is stimulated under conditions of neurosecretory stress and hypothalamic-pituitary-adrenal (HPA) axis activity. Pituicytes also show mitotic activity following dehydration [9]. The increased number of mitoses in pituicytes of the pars nervosa is greatly augmented if rats are simultaneously injected with lithium, a cation used as an osmotic stressor [10, 11]. In the course of studying the mitogenic effects of lithium in the pars nervosa, preliminary observations noted mitoses in the median eminence of the hypothalamus, suggesting a hypothalamic localization of a population of pituicytes, which is the subject of the current investigation.

Experimental Procedures

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Nathan Kline Institute. Lewis rats of either sex ($n = 51$) were kept in hanging shoebox type plastic cages with corncob bedding. Laboratory Rodent Diet 5001 chow (Purina, Scott's Distribution, Hudson, NH) was available ad libitum. The rats were used when 7–10 weeks old (180–260 grams). Rats ($n = 25$) were given an intraperitoneal injection of isotonic lithium chloride (LiCl; 0.15 M) in water, 2 ml per 100 g body weight for three consecutive days. Water contained in the injections served as hydration, as described by Kawamoto and Kawashima [12]. A control cohort of rats ($n = 26$) did not receive any experimental procedures.

Rats were killed by exsanguination while under CO₂ anesthesia. Serum lithium levels were assessed by preparing blood taken from the inferior vena cava at the time of sacrifice. Lithium was assayed by atomic absorption spectrophotometry as described previously [10, 11]. The brain was removed quickly, placed into Bouin's fluid and the base of the skull was immersed in Bouin's fluid to fix the pituitary in situ for 24 h at 22°C. The brain and pituitary were embedded in paraffin, serially sectioned at 5 μ m, and stained with hematoxylin and eosin as described previously [10, 11]. The level of the mediobasal hypothalamus which presented the largest area of median eminence was studied. Tissue sections were examined at 400 $\times$ magnification and mitoses throughout the median eminence were counted as described previously [11]. This approach obviated concerns regarding tissue selection bias [11]. A profile was considered a mitotic figure when the nuclear membrane was absent and multiple chromosomes were visualized. The area of the median eminence and the neural lobe was determined with the aid of an ocular reticule, calibrated by a stage micrometer.

Results and Discussion

Lithium levels were significantly increased in experimental rats (0.375 ± 0.07 mEq/L) compared to control untreated rats (0.17 ± 0.05 mEq/L) (t test; $P < 0.001$) as described previously [10, 11]. Coronal sections through the hypothalamus of normal control rats revealed the median eminence at the base of the third ventricle. The first layer was composed of pleomorphic cuboidal or columnar ependymal cells, the tanycytes. Ventral to this layer there was a poorly demarcated layer of similar cells, constituting the subependymal layer. The deep ventral aspect of many of the ependymal and subependymal cells tapered off into a process that could often be followed into the subjacent fiber and

reticular layers, but they could not be followed into the most distant layer, the palisade layer. The fiber and reticular layers were not easily distinguished from each other. These layers contained a small number of glial nuclei, round, oval or reniform, whose cell borders were not apparent. Vacuolar pathology was not observed in the median eminence of normal rats. However, round vacuole-like structures, often termed cisternae, were present in control rats at both lateral margins of the median eminence, in the regions of the tuberohypophyseal sulci as described previously [13, 14]. These cisternae within the median eminence did not contain nuclei and were not quantified. The palisade layer of the median eminence was readily identified by its uniformly striated appearance. It was covered ventrally by the capillary network of the hypophyseal portal vessels which were partially separated from the subarachnoid space by the pars tuberalis of the pituitary.

Mitotic figures were not detected in the median eminence within any of the control rats ($n = 26$). In contrast, mitotic figures were observed in 12 out of 25 (48%) rats that underwent the experimental procedure. The incidence of mitoses in the median eminence was lower than the incidence of mitoses in the pars nervosa as described previously [10, 11]. This difference in mitotic activity can be partially explained by the size of the area of analysis for the median eminence (approximately 0.3 square mm) and pars nervosa (approximately 0.8 square mm).

Mitotic figures were located in the ependymal (tanycyte) layer of all 12 rats that displayed mitoses (Fig. 1). Mitotic figures were also observed with a lesser degree of frequency within the subependymal layer (one rat), within the fiber-reticular layers (8 rats), and within the palisade layer (one rat). Seven rats had mitoses in the pituitary stalk, but this number is an underestimate compared to the median eminence because the stalk was not present in every tissue section in this study. Mitoses in the fiber, reticular, and palisade layers of the median eminence and those found in the pituitary stalk resembled pituicyte mitoses described previously in the pars nervosa of the pituitary [10, 11]. The mitotic cells were large and round with abundant cytoplasm, and the chromosomes were well separated (Fig. 1).

There are many ways to measure the proliferation of cells within the brain and pituitary, but we believe that direct morphological observation and enumeration of mitotic figures is highly advantageous within this experimental paradigm. For example, increased DNA synthesis in the neurohypophysis is not always accompanied by detectable mitoses [15], so this measure can be equivocal within the hypothalamus and pituitary. Pituicytes undergoing mitosis are relatively large and easily identifiable by hematoxylin and eosin staining in tissue sections containing the basal hypothalamus, median eminence, and pituitary. Direct observation of mitoses enables the observer to place the dividing cell into its histologic niche. This procedure was relatively simple in the uniform structure of the pars nervosa of the pituitary [10, 11], but was more difficult in the complex laminar structure of the median eminence. A few mitotic figures were found in the fiber, reticular, and palisade zones in which glial cells were relatively uncommon. These mitoses were similar in appearance to the mitotic pituicytes of the pars nervosa, adding to the evidence that the mitotic cells in the median eminence were actually pituicytes. The majority of the mitotic cells were in the ependymal (tanycyte) layer. These data suggest that pituicytes were present among the densely packed ependymal cells, and to a lesser degree in the less densely packed fiber, reticular, and palisade zones. Although the functional significance of the laminar differences in mitotic figures remains unknown, we posit that the presence of pituicytes is highest in the ependymal layer compared to the other layers of the median eminence, and that the ependymal layer may be the most dynamic in terms of its response to stimuli, including osmotic stressors as well as alterations within the HPA axis. Light microscopic observations in the current report are supported by ultrastructural evidence that some ependymal cells have astrocytic morphology, and by light and immunoelectron microscopic identification of

discrete populations of pituicytes that express the glial markers glial fibrillary acidic protein (GFAP) and S-100 [6, 12, 16]. The lower incidence of pituicyte mitoses in the median eminence compared to the pars nervosa of the pituitary is likely related to the fact that the median eminence contains many nerve fibers en route to the pars distalis and unrelated to the pars nervosa. In summary, mitotic figures were observed in the median eminence, indicating that specialized glial cells, pituicytes, are likely to exist within the median eminence, and may play a role in the active process of neurosecretion following stimuli such as osmotic stress.

Acknowledgments

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Fig. 1. Mitotic figures in the median eminence and infundibular stalk of rats treated with lithium for 3 days. Sections were 5 μm thick, stained with hematoxylin and eosin. **a** The vertical slit is the third ventricular lumen. The median eminence is on the left. The ependymal (tanycyte) layer is crowded with cells, one of which displays mitosis (*arrow*). **b** The vertical slit is the lumen of the infundibular stalk lined by ependyma on both sides. Six mitotic cells can be seen in the ependymal layer in this one microscopic field. **c** Mitosis in a glial cell (*arrow*), thought to be a pituicyte, at the junction of fiber-reticular and palisade layers of the median eminence. The *dark areas* on the *left* are capillaries of the portal circulation filled with erythrocytes. **d** Mitosis in a glial cell (*arrow*), thought to be a pituicyte, in the subependymal layer of the median eminence. Scale bar a–d: 50 μm