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Distribution of osmoregulatory peptides and neuronal-glial configuration in the hypothalamic magnocellular nuclei of desert rodents

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ABSTRACT

The desert rodents *Psammomys obesus* and *Gerbillus tarabuli* live under extreme conditions and overcome food and water shortage by modes of food and fluid intake specific to each species. Using immunohistochemistry and electron microscopy, we found that the hypothalamic magnocellular nuclei, and in particular, their vasopressinergic component, is highly and similarly developed in *Psammomys* and *Gerbillus*. In comparison to other rodents, the hypothalamus in both species contains more magnocellular VP neurons that, together with oxytocin neurons, accumulate in distinct and extensive nuclei. As in dehydrated rodents, many magnocellular neurons contained both neuropeptides. A striking feature of the hypothalamic magnocellular system of *Psammomys* and *Gerbillus* was its display of ultrastructural properties related to heightened neurosecretion, namely, a significant reduction in glial coverage of neuronal somata and dendrites in the hypothalamic nuclei. There were many neuronal elements whose surfaces were directly juxtaposed and shared the same synapses. Their magnocellular nuclei also showed a high level of sialylated isoform of the Neural Cell Adhesion Molecule (PSA-NCAM) that underlies their capacity for neuronal and glial plasticity. These species thus offer striking models of structural neuronal and glial plasticity linked to natural conditions of heightened neurosecretion.

1. Introduction

Osmotic regulation in mammals is in part under the control of the neurohormones vasopressin (VP) and oxytocin (OT) that are produced by magnocellular neurons in the hypothalamus constituting the hypothalamo-neurohypophysial system (HNS). The somata and dendrites of the neurons accumulate mainly in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus and their axons project to the neurohypophysis or neural lobe (NL) of the pituitary. Over the past 50 years, the rat HNS has served as model for the study of most aspects of neurosecretion and many electrophysiological and cell biological properties of the neurons have been defined under different physiological conditions. Moreover, it is well established that when these neurons are strongly or chronically solicited, their major synaptic inputs controlling their activity as well as associated glial astrocytes, undergo a remarkable morphological plasticity [1].

Desert rodents appear as unique models for the study of neurosecretion and of HNS peptides, in particular. They have an impressive ability to adapt to extreme osmotic environments. Heat and aridity, water deprivation, high insolation and convection with consequent high evaporation characterize their biotope. For desert mammals, hydro-osmotic regulations are more important than for any other mammal because of their dry environment and the high salt content of their diet. Several physiological

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studies have described the surprising renal concentrating power displayed by desert rodents [2], which is linked to the particular structure of their kidneys with their long renal papilla [3,4]. In addition, it has been reported that the concentration of VP in the NL and plasma of these species is significantly higher than in laboratory rats [5–7]. Such characteristics allow such animals to survive prolonged periods of drought and thus inhabit extremely arid areas successfully.

Two species that exist in particularly difficult conditions of water shortage and state of highly chronic dehydration in the Algerian desert are *Gerbillus tarabuli* and *Psammomys obesus*. The former is nocturnal and granivorous, whereas the latter is diurnal and herbivorous. Both species obtain water from their food, eating leaves and stems of chenopod plants with high salt content; they rarely drink. Because of such drastic conditions, it is highly probable that the HNS of these species displays particular characteristics linked to highly activated neurosecretion. Nevertheless, because of difficulties in the capture and subsequent maintenance of these animals in the laboratory, relatively few studies have been conducted until now.

In the present study, we obtained sufficient animals to allow morphological and immunocytochemical analyses of their VPergic and oxytocinergic systems. We also used electron microscopy to determine whether their hypothalamic magnocellular nuclei display any of the characteristics typical of the activity-dependent morphological remodeling of the HNS until now described in laboratory rodents, like the rat and the mouse [8,9]. Finally, we determined whether their HNS expresses plasticity-related glycoproteins, and in particular, the highly sialylated isoform of the Neural Cell Adhesion Molecule (PSA-NCAM) that our earlier studies [9–11] showed to be indispensable for neuronal and glial transformations, at least in the rat and mouse HNS under different stimuli of neurosecretion.

2. Materials and methods

2.1. Animals

Wild adults from male and female *P. obesus* and *G. tarabuli* species were captured in the desert (near Beni Abbès, Southwest of Algeria) from March to June. Captured animals were housed in a research facility (Unité de Recherches sur les zones Arides de Beni Abbès) for three days before sacrifice. During the 3-day captivity, animals were exposed to a temperature of 22 ± 2 °C and a light-dark cycle (12L-12D) and fed *ad libitum* with chenopod plants (*Psammomys*) and barley seeds (*Gerbillus*). Seventeen *G. tarabuli* (35–50 g) and 15 *P. obesus* (80–100 g) were used for these studies. All manipulations conformed to local and international guidelines on ethical use of animals.

2.2. Tissue fixation

Animals were deeply anesthetized with urethane (1.5 mg/kg), injected intracardially with heparin and then perfused with fixative. One group of animals was perfused with solution composed of freshly prepared 4% paraformaldehyde and 0.1% glutaraldehyde in sodium phosphate buffer (0.1 M, pH 7.4, 200–300 ml during 20 min, at room temp). After postfixation in 4% paraformaldehyde overnight (4 °C), brains were removed and cut on vibratome to obtain serial frontal slices (about 30–50 μm) which underwent immunolabeling for light microscopy.

Another group of animals was perfused with a freshly prepared solution of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4; 200–300 ml during 20 min, room temp). Following perfusion, brains were removed and postfixed for 2 h in the same solution. Blocks containing the SON were then dissected from frontal slices of the brain. These tissues were prepared further for electron microscopy.

2.3. Immunohistochemistry

For single immunolabeling, standard immunofluorescence and immunoperoxidase techniques were performed on free-floating sections of brains according to procedures described in detail in earlier studies [12–14]. Briefly, after rinsing in Tris-buffered saline (TBS), sections were treated with casein (0.5% in PBS) for 1 hr to block non-specific sites. They were then incubated for 24–48 hr at 4 °C with: (i) polyclonal rabbit antibodies against either VP (diluted 1:5000–1:10 000) or against OT (1:400–1:1000) [15] (gift of A. Burlet); or, (ii) monoclonal mouse IgM antibody that specifically recognizes PSA on NCAM (diluted 1:2000–1:8000) (gift of G. Rougon), (see [16] for production and specificity). Affinity-purified anti-rabbit or anti-mouse immunoglobulins (IgG) conjugated to fluorescein isothiocyanate (FITC, Biosys) (diluted 1:400, 2 hr, at room temperature) or FITC-conjugated anti-mouse IgM (diluted 1:100) were used. For immunoperoxidase, after incubation with anti-rabbit or anti-mouse Igs (diluted 1:200 or 1:400 respectively), sections were incubated with rabbit peroxidase-antiperoxidase (PAP, Dako) complexes (diluted 1:100) or with anti-mouse IgM conjugated to horseradish peroxidase (HRP, 1:50). Peroxidase reaction product was revealed either with 3, 3′-diaminobenzidine (DAB) and 0.01% H_2O_2 or with the more sensitive glucose oxidase-nickel-DAB method.

In some cases, double immunofluorescence was performed on free-floating sections incubated for 48 hr at 4 °C in mixtures of primary antibodies containing the polyclonal rabbit IgG anti-VP (diluted 1:5000) and monoclonal mouse antibodies specific for OT-linked neurophysin (OT-Np; diluted 1:500) [17] (gift of H. Gainer). After careful rinsing, the sections were incubated for 2 hr at room temperature in a mixture of fluorescent conjugates. Rabbit FITC-conjugated anti-rabbit Igs (diluted 1:400) were used to identify VP immunoreactivity, whereas anti-mouse Igs conjugated with Texas Red (TR) (Biosys, diluted 1:500) were used to visualize OT-Np immunoreactivities. The preparations were examined with light microscopy (Leica DMR) using bright field optics for HRP-containing sections and epifluorescence with appropriate filters for FITC- or TR-treated sections.

Controls: controls included omission of primary antibodies and incubation in normal rabbit serum or with irrelevant secondary antibodies. These sections did not show any labeling.