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Ependymal tanycytes projecting to the ventromedial hypothalamic nucleus as demonstrated by retrograde and anterograde transport of HRP

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The neural associations of the ventromedial (HVM) and lateral hypothalamic nuclei (HL) in mammals have been intensively studied in view of the role these hypothalamic centers are supposed to play in the neural control of ingestive behavior, besides a variety of other behavioral functions. A number of studies both with degeneration and intracellular transport techniques have appeared, reporting in detail the efferent and afferent connections of the two hypothalamic nuclei. These studies have all been concerned with the neural relationships of the entire nuclear complexes. Recent behavioral studies, however, have shown that local stimulation or lesioning of subdivisions of HL and HVM result in different behavioral effects. As these results suggest different anatomical relations for various parts of HVM and HL, this led us to an analysis of HVM and HL connections on a subnuclear level.

As part of this analysis we will present here the results of experiments that demonstrate cellular connections of tanycytes in the lateral ventricular wall to specific parts of the HVM.

The evidence is based on 8 horseradish peroxidase injections in various parts of the HVM and 3 in the periventricular ependyma (Fig. 1A–E). All experiments were performed on male albino Wistar rats weighing 300 g. Glass micropipettes with tip diameters between 15 and 30 μm were filled with a 10–15% HRP (Serva or Sigma, type VI) solution in 0.01 M NaCl and placed stereotactically according to the atlas of König and Klippel. A positive DC current of 1.5–2.0 μA, half time on – half time off, was applied for 15 min total on-time. After iontophoresis the pipette was left in situ for at least 10 min to prevent loss of HRP in the electrode track. Following survival times of 2 days the animals were reanaesthetized and perfused with warm saline followed by a 1% paraformaldehyde and 1.5% glutaraldehyde solution in phosphate buffer (0.1 M; pH = 7.4) containing 4% sucrose. After fixation overnight the brains were cut at 40 μm on a freezing microtome and every second section was stained for HRP according to the DAB method of Graham and Karnovsky, the benzidine di-HCl procedure of Mesulam and Rosene, or the DAB modification of Malmgren and Olsson.
Figure 1. A–E: series of transverse sections through the ventromedial hypothalamic nucleus at levels given in the bottom chart. A, B and C: HRP injection spots in the ventromedial nucleus that resulted in retrograde labeling of ependymal tanyocytes. D and E: injections are indicated in the ventricular wall. The anterior injection at D resulted in anterograde, HVM-directed fiber labeling. More posterior injections such as in E did not produce labeling of tanyocytes. F: photomicrograph of the injection spot drawn in C with retrogradely labeled tanyocytes. Bar = 200 μm. G: photomicrograph of labeled tanyocytes. Bar = 50 μm. H: photomicrograph of the HRP injection drawn in D with anterogradely labeled tanyocytes projecting into the HVM. Bar = 200 μm. Abbreviations: AM, amygdala; AR, nucleus arcuatus; CAI, capsula interna; F, fornix; FMT, fasciculus mammillothalamicus; HDM, dorsomedial hypothalamic nucleus; HL, lateral hypothalamic area; HPV, periventricular hypothalamic nucleus; HVM, ventromedial hypothalamic nucleus; TO, tractus opticus; TV, ventral thalamus; VIII, third ventricle; ZI, zone incerta.
A survey of the results is given in Fig. 1. HRP injections in the central (Fig. 1A) or medial (Fig. 1B, C) aspects of the anterior two thirds of the HVM resulted in retrograde labeling of clusters of ependymal cells in the adjacent ventricular wall. Peroxidase deposits in other divisions of the HVM failed to produce such retrograde cell labeling. The same holds true for HRP injections in other hypothalamic areas such as lateral and dorsolateral hypothalamic nuclei.

The connection of periventricular cells restricted to medial and anterior HVM areas was also demonstrated by anterogradely transported HRP following injections in the periventricular ependyma (Fig. 1D-E). The peroxidase injection as illustrated in Fig. 1D resulted in heavy anterogradely labeled cellular processes to the HVM, whereas such injections at more posterior levels produced only labeling of short neurites that were restricted to the periventricular area. These results indicate the presence of ependymal cells projecting to specific areas in the HVM.

According to their structure the ependymal cells described here most likely should be regarded as what was termed before as tanyocytes. This type of liquor-contacting cells is abundantly present in the median eminence where they are considered to be involved in neurohypophysial processes. Tanyocytes situated more dorsally in the lateral wall of the third ventricle have previously been noticed and described by Millhouse in Golgi material.

Several theories can be found in the literature with respect to the functional character of tanyocyte connections. Whatever their precise mode of action may be, it seems likely that these cells influence the exchange of substances between cerebrospinal fluid (CSF) and specific parts of the HVM, or more likely to its vasculature, since Millhouse has mentioned tanyocyte fiber endings on small hypothalamic blood vessels.

The assumption that tanyocytes act as intermediaries between CSF and HVM received further support by some preliminary experiments in which we injected 0.5 µl, 4',6-diamidino-2-phenylindol 2HCl and premuline (DAPI-Pr) in the lateral ventricle. After two days survival time these injections resulted in fluorescent anterograde labeling of tanyocyte cell processes to the HVM divisions described above.

Whether this CSF–HVM connection plays an active role in feeding control mechanisms remains unclear. It is reported, however, that an increase of CSF insulin levels in the baboon reduces the food intake and body weight, while insulin can have dramatic effects on HVM cellular activity. The CSF–HVM tanyocyte connection reported here suggests a possible although still unknown role in the interaction between CSF and HVM cells involved in feeding control. Current experiments in which we use radioactive insulin are carried out to elucidate this problem.


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