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Organotypic culture of central histamine neurons

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Organotypic cultures of histaminergic tuberomammillary (TM) neurons were grown using explants obtained from newborn rats. The cultures were examined after immunohistochemical localization of the histamine synthetic enzyme, L-histidine decarboxylase (HDC). The morphological properties of the somata, dendrites and axons of HDC-immunoreactive TM neurons in organotypic culture were virtually indistinguishable from those seen in situ. Extensive plexuses of HDC-immunopositive axons, including growth cones, were seen within the hypothalamus, the plasma surrounding the explant and co-cultured hippocampus. Organotypic cultures of TM histamine neurons, and co-cultures with their targets, provide a useful model system for studying several aspects of central histaminergic neurobiology.

Organotypic cultures of central nervous system explants are powerful tools for studying the structure and function of isolated portions of the brain. Numerous transmitter-specific explant culture systems have been developed[2,9,10,17,26]; the present report describes organotypic culture of central histamine neurons.

The neurons of the tuberomammillary (TM) nucleus of the caudal hypothalamus exhibit immunoreactivity to both histamine[12,22] and its synthetic enzyme L-histidine decarboxylase (HDC)[13,25]. At present, TM neurons appear to be the sole histaminergic neurons in the rat brain. TM neurons innervate widespread regions of the neuraxis including the cerebral cortex and hippocampus[11,24,25]. In the present study, the ability of the HDC-immunoreactive neurons of the TM nucleus to survive in organotypic culture as well as to innervate co-cultured hippocampus was investigated.

Eleven newborn rats (Osborne–Mendel, day 0) were sacrificed by decapitation and the tuber cinereum[16] and hippocampus dissected free under aseptic conditions. Coronal sections (400 μm thick) were prepared using a tissue chopper, and co-cultures of the TM region and hippocampal formation prepared using methods similar to those described by Gähwiler[4]. Cultures were embedded in chicken plasma on a glass coverslip and grown in medium containing 25% horse serum, 50% basal medium Eagle (with Earle's salts), 25% Hanks' balanced salt solution and 0.65% glucose. Cultures were incubated at 36 °C in a roller drum rotating at 10 revolutions/h. The nutrient medium was replaced twice weekly. Cultures were maintained for 15–37 days, after which they were processed for HDC immunohistochemistry.

HDC immunohistochemistry was carried out using polyclonal antibodies whose specificity has been previously documented[25]. Co-cultures were washed for 1 h in Hanks' balanced salt solution, fixed at room temperature for 1 h in 4% paraformaldehyde in 0.12 M phosphate buffer (pH 7.3) followed by three 20-min washes in phosphate-buffered saline (PBS). Coverslips with cultures attached to them were placed in multi-well plates and incubated under sterile condi-

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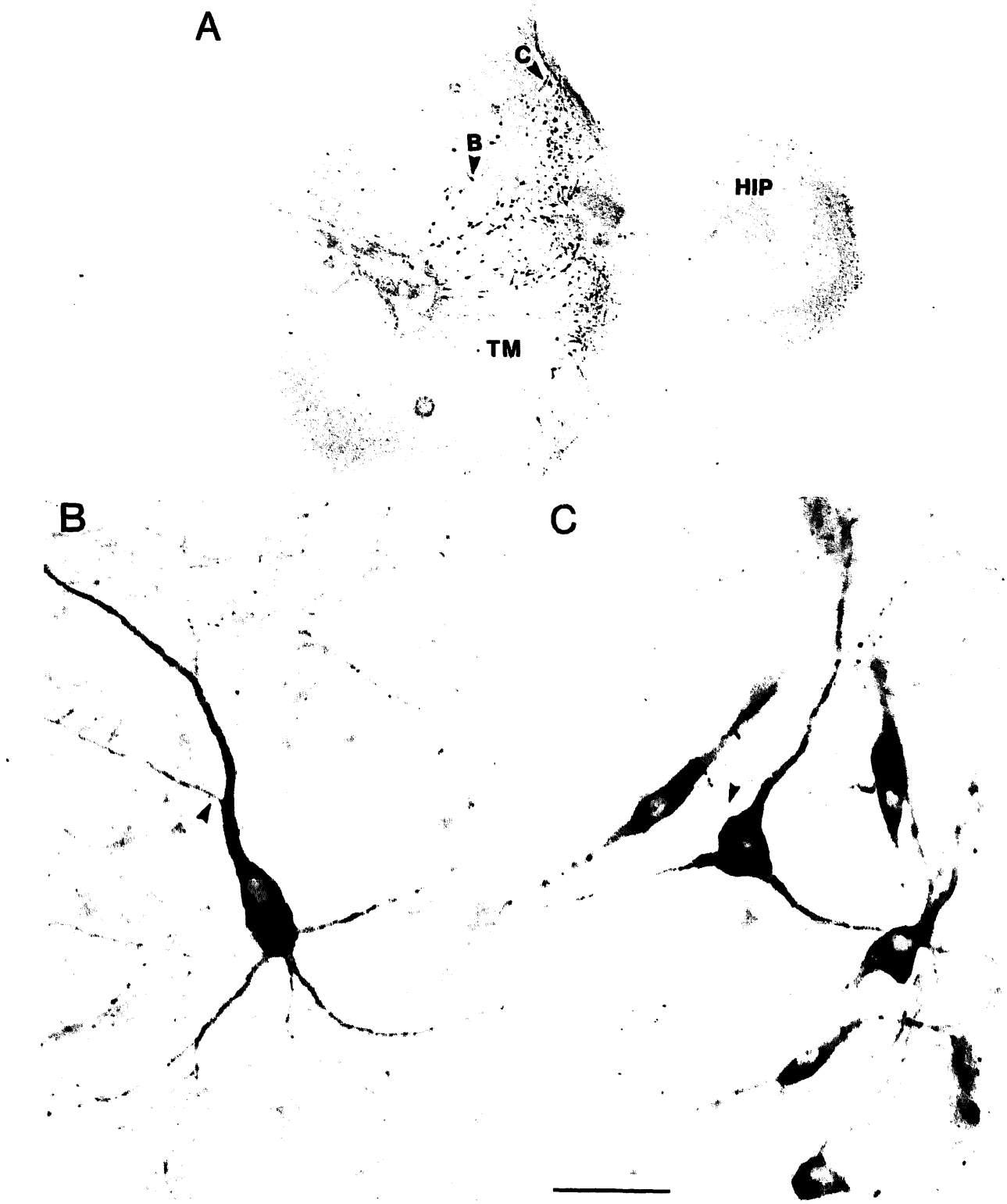

Fig. 1. A: co-culture of tuberomammillary nucleus (TM) and hippocampus (HIP) grown for 29 days and processed for HDC immunoreactivity. HDC-immunoreactive somata can be seen to be distributed bilaterally throughout the TM region. Arrowheads B and C point to neurons shown at higher magnification below. B: morphology of an HDC-immunoreactive TM neuron with the axon (arrowhead) emerging from a prominent dendritic emission cone. C: morphology of an HDC-immunoreactive TM neuron with the axon (arrowhead) emerging from the soma. Bar in A = 1 mm; in B and C = 50 μm.

tions in primary antibody (rabbit anti-HDC) at a dilution of 1:10,000 with 10% normal goat serum and 0.3% Triton X-100 in PBS for 72 h at 4 °C with agitation. The cultures were processed for standard avidin–biotinylated horseradish peroxidase immunohistochemistry as previously described[16], with the exception of the addition of 10% normal goat serum. Two cultures were processed for nickel ammonium sulfate enhancement of the diaminobenzidine reaction product[18] to enhance visualization of immunoreactive axons.

In 9/11 cases, HDC-immunoreactive neurons were found in the cultured hypothalami (Fig. 1A). Although non-histaminergic neurons likely survived the culture procedure as well, the present observations are limited to HDC-immunoreactive elements. HDC-immunoreactive somata were round or oblong and multipolar, with 2–3 primary dendrites (Fig. 1B,C). The polar dendrites of HDC-immunoreactive TM neurons frequently emerged from a large emission cone (Fig. 1B). Dendrites, which also exhibited HDC immunoreactivity, were sparsely branching and extended for a considerable distance from the soma; immunoreactive dendritic spines were not seen. In several cases, the axon could be identified by following its retrograde course from a fiber plexus in the surrounding plasma or co-cultured hippocampus. Most commonly, the axon emerged from the trunk of one of the polar dendrites (Fig. 1B), as has been noted in situ[23,27]; less frequently, the axon arose directly from the soma (Fig. 1C). No HDC-immunoreactive perikarya were found in cultured hippocampi.

HDC-immunoreactive axons were extensively distributed throughout the hypothalamus (Fig. 2A), and many of these axons exhibited terminal growth cones (Fig. 2A). HDC-immunoreactive axons were also found extending into the plasma surrounding the explant, and the density of axons within this plasma was remarkable (Fig. 2B). In 7/9 cases where HDC-immunoreactive cell bodies were recovered, HDC-immunoreactive fibers emanating from the hypothalamus were also found in the co-cultured hippocampus (Fig. 2C); some of these axons exhibited terminal growth cones. HDC-immunoreactive fibers could be followed across the gap between the hypothalamus and hippocampus and into the hippocampus proper, although in no case was the degree of hippocampal

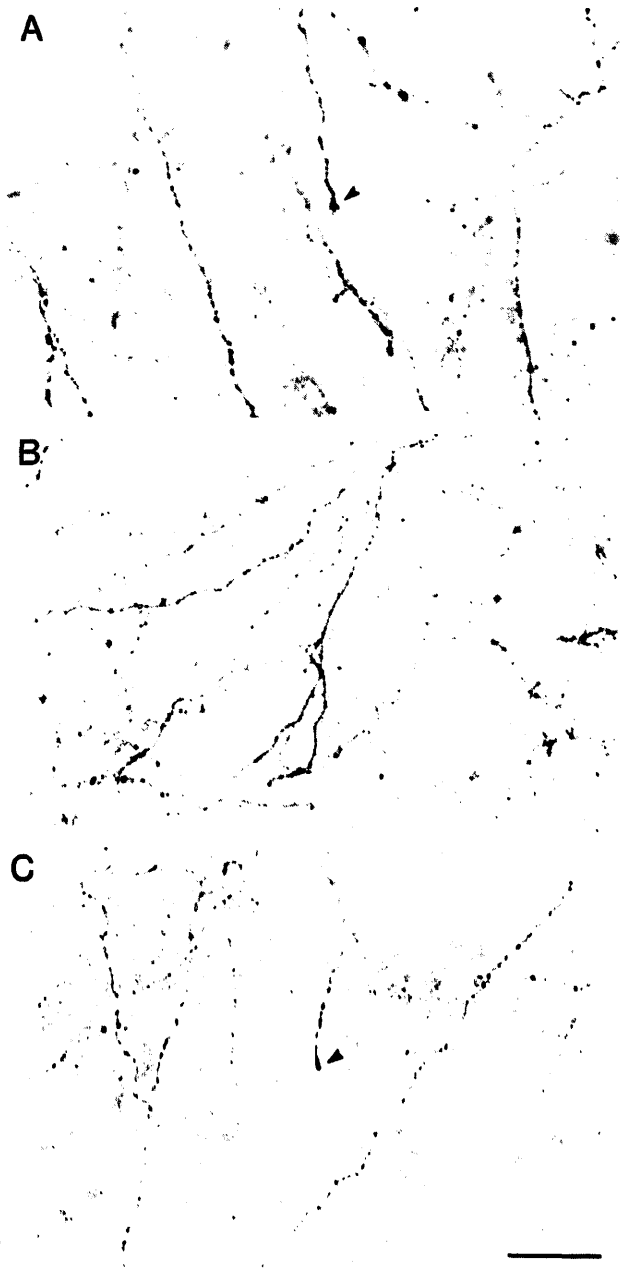

Fig. 2. HDC-immunoreactive axons in the hypothalamus (A), the plasma (B) and the hippocampus (C). Note the presence of numerous varicosities and growth cones (arrowheads in A and C). Bar = 50 μm for A–C.

innervation extensive. HDC-immunoreactive fibers were usually varicose (Fig. 2A–C), and occasional fibers displayed bulbous enlargements. HDC-immunoreactive axons within the hypothalamus appeared to consist of a mixture of very thin and moderate diameter fibers (Fig. 2A), as were those which extended into the surrounding plasma (Fig. 2B). In contrast, HDC-immunoreactive fibers within the co-cul-

tured hippocampus were almost invariably of fine diameter (Fig. 2C). There was no obvious topographical distribution of HDC-immunoreactive fibers innervating the co-cultured hippocampus.

HDC immunoreactivity can be detected in the fetal rat brain as early as embryonic day 16[15]. Since in the present study explants were harvested from newborn rats, the presence of HDC immunoreactivity in these cultures probably represents maintenance of a previously expressed trait, rather than development of the phenotype in vitro. It is unlikely that TM neurons proliferated in these explant cultures since HDC-immunoreactive neurons undergo their final mitotic division around day 16 of embryonic life[15]. However, it is fairly certain that at least some of the HDC-immunoreactive axons found in the co-cultured hippocampi represent a sprouting process of TM axons in vitro for the following reasons: (1) HDC-immunoreactive somata in these co-cultures were only found within the hypothalamus; (2) HDC-immunoreactive axons could be traced from TM neurons in the hypothalamus to the hippocampus; and (3) HDC-immunoreactive growth cones were seen within the co-cultured hippocampi. There remains the possibility that some of the HDC-immunoreactive axons found in the hippocampus represent HDC-positive neurites which might be detected even in isolated hippocampal explants. Experiments are underway to examine this possibility. At present, it is unclear as to whether the growth of HDC-immunoreactive axons is a response to the culture procedure per se, or results from the fact that the tissue was harvested very early in the postnatal period, or both, as the timing of axonal growth of histaminergic TM neurons is unknown. Furthermore, while ingrowth of HDC-immunoreactive axons into co-cultured hippocampi was observed, conclusions as to whether synaptic contacts are made awaits the results of ultrastructural and functional studies.

In many neuronal systems, the presence of appropriate target tissues and/or factors derived from them enhances survival and outgrowth of axons in culture[1,6]. Whether TM neurons require their natural targets for axonal growth in culture is not clear from the present data, as both the hippocampus and the hypothalamus are major targets for TM axons[11,21]. Nonetheless, the observation that HDC-immunoreactive axons in the hypothalamus and surrounding plasma were of varying diameter while those found within the hippocampus were only of fine caliber suggests some selectivity of axonal morphology which is target dependent. Assuming that axonal diameter correlates with conduction velocity, these data are consistent with recent electrophysiological results which demonstrate that cortically projecting TM neurons have exceedingly slow conduction velocities[14] while at least some TM neurons projecting to hypothalamic targets have relatively rapid conduction velocities[8].

There are numerous potential applications for organotypic culture of histamine neurons. For example, it has been shown that bath-applied histamine decreases a calcium-activated potassium conductance in hippocampal pyramidal cells in vitro[7]. Using co-cultures of the histaminergic neurons of the TM nucleus and the hippocampus as described herein, it may now be possible to test directly whether this phenomenon is biologically relevant, as has been recently accomplished for the cholinergic input from the septum[5]. Organotypic cultures have also been used to examine the effects of various pharmacological and electrophysiological treatments upon levels of neurotransmitters and synthetic enzymes in central neurons[3]. As TM neurons have been shown to contain a plethora of co-localized transmitters and related enzymes[11,19,20], such experiments may provide new insights into the biochemical control of their transmitter plurality.

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