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Neuronal and glial localization of acetylcholinesterase and GABA transaminase in organized cultures of developing rat spinal cord

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Summary. Acetylcholinesterase (AChE) and γ-aminobutyrate transaminase (GABA-T) were localized in organized cultures of rat embryo spinal cord. AChE was seen in neurones of the ventral horn and intermediate nucleus and in the dorsal horn neuropil. AChE was also present on the soma membrane of neuroglia, even in areas devoid of AChE-stained neuronal processes. GABA-T activity was present chiefly in dorsal horn neurones, and was absent from neuroglia.

Acetylcholinesterase (AChE) and γ-aminobutyric acid aminotransferase (GABA-T) are enzymes whose function is the inactivation of the neurotransmitters acetylcholine and GABA respectively. Both enzymes are trans-synaptic, i.e. they are present both in presynaptic neurones containing the transmitter and in the post-synaptic membrane[1,2]. Thus these enzymes serve as markers for the existence, or the potential for formation, of functional synapses where acetylcholine and GABA are transmitters.

Organotypic cultures were prepared from transverse sections of spinal cord from Hooded Lister rat embryos of 14–17 days' gestation as described elsewhere[3,4] and were maintained for 7–28 days. The anatomical arrangement of cultures was similar to that seen in spinal sections. For acetylcholinesterase staining, intact cultures were fixed in 2% buffered formol calcium for 10 min at 4 °C. The reaction was carried out using the thiocholine technique[5] as adapted by El Badawi and Schenk[6]. The reaction was carried out in semi-solid phase in some experiments by making up the reaction media in 3% ion agar No.2. The preparations were counterstained with Giemsa stain. Specificity of the AChE reaction was tested by the inclusion of 10^{-4} M iso-OMPA, an inhibitor of pseudocholinesterase. GABA-T was demonstrated using the procedure of Hyde and Robinson[7] on unfixed cultures at an incubation temperature of 37 °C. Controls for each technique were prepared by the omission of the substrate.

Enzyme reaction products reached their maximum intensity after 10 days in vitro for AChE and 21 days in vitro for GABA-T. The positions of groups of neurones stained with each method was noted in 20–30 cultures. Neurones were all stained evenly in the cytoplasm with both methods. Heaviest deposits of AChE were seen over large ventral horn neurones[8] (fig. 1,a). Lighter deposits were seen in the dorsal pole of the explant as a diffuse staining of the neuropil (fine axonal and dendritic processes) and of some small neurones (fig. 1,b). AChE was also present in the intermediate nucleus and commissural bundles connecting the two halves of the cord. Glial cells were easily distinguished from other cell types on morphological criteria[9,10], having very fine, copiously branched cytoplasmic processes and a small phase-dull cell body. The same cells were heavily stained on the soma membrane even in areas of the cultures devoid of AChE-stained neurones or their processes (fig. 1,c and d). AChE reaction product was seen in all of the above sites when iso-OMPA was included in the medium. Diffusion of reaction product did not take place since no change in its distribution was seen either in neurones or glia when the reaction was carried out in agar.

GABA-T was observed in large numbers (up to 50 per

culture) of medium-sized neurones in the dorsal half of the explant, many of which gave rise to fine, varicose axons (fig. 2, a and b). Some of these travelled distances of up to 1 mm around the perimeter of the explant (see Guillery et al.[8] for a detailed anatomical study of this type of dorsal neurone). Ventral horn cells were very weakly stained. GABA-T staining was not seen, either in the cytoplasm or in the soma membrane of glial cells in 23 culture preparations.

The distribution of AChE in rat organotypic cultures agrees well with its distribution in the adult rat spinal cord in situ[11]. This report provides evidence that AChE is not only located in the cytoplasm of neurones in the spinal cord in culture in the same areas as those seen in the cord in situ, but is also seen on the soma membrane of neuroglia.

GABA-T was concentrated in neurones in the dorsal half of the cord as predicted from biochemical measurements in vivo[12]. This region also contains highest concentrations of GABA[13], glutamate decarboxylase[14], high-affinity GABA receptors[15] and the highest capacity for GABA uptake[16]. In contrast to AChE-stained cultures, no GABA-reaction was seen in glial cells. Since the cultures are prepared from the developing CNS, it is possible that most neuroglia therein are immature and have not acquired the capacity to produce GABA-T often seen in their adult counterparts[17]. However, neuroglia cultured from rodent spinal cord are unable to accumulate GABA-(H^3)[18] unlike those cultured from brain[19]. The absence of staining for GABA-T in spinal glia may further indicate their inability to metabolize GABA.

The presence of AChE in neuroglia suggests that they are capable of inactivating acetylcholine. The presence of muscarinic receptors[20] and membrane responses to acetylcholine[21] but not to other transmitters[22] have been demonstrated in cultured glial cells. These observations indicate a possible role for acetylcholine in the regulation of glial physiology.

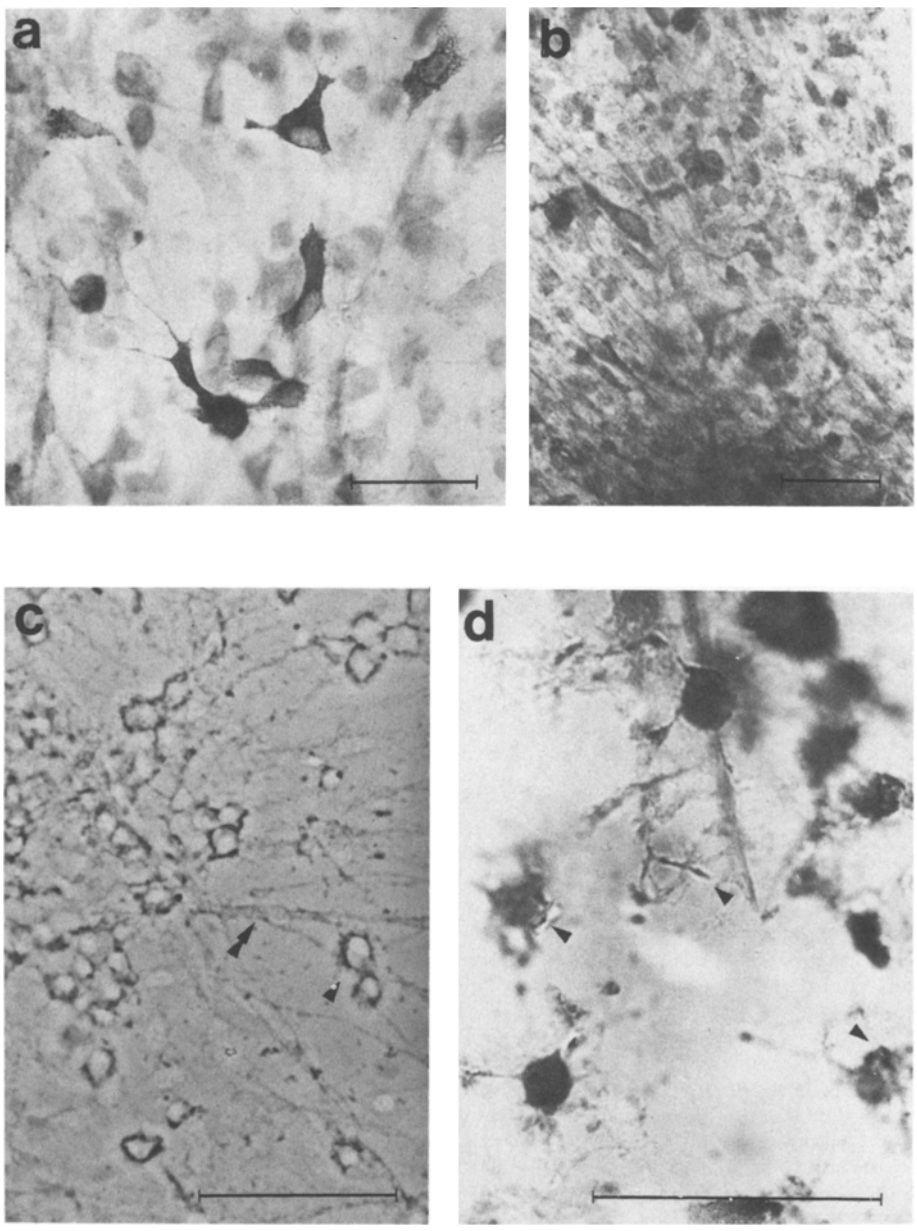

Figure 1. AChE cytochemistry in spinal cord cultures (10 days in vitro). *a* Immature ventral horn neurones showing AChE staining in the cytoplasm. *b* Weaker staining of spindle-shaped neurones and nerve fibers in the 'dorsal horn'. *c* AChE staining on soma membrane of cells with glial morphology (arrowed). Note unstained nerve fibers (double arrow). *d* Glia-like cells with AChE deposits on somata and processes (arrowed). Staining was seen in all of the above sites in preparations incubated with iso-OMPA. *a, b, d* Counterstained with Giemsa. *c* AChE stain alone with phase contrast. Calibration bars indicate 50 µm.

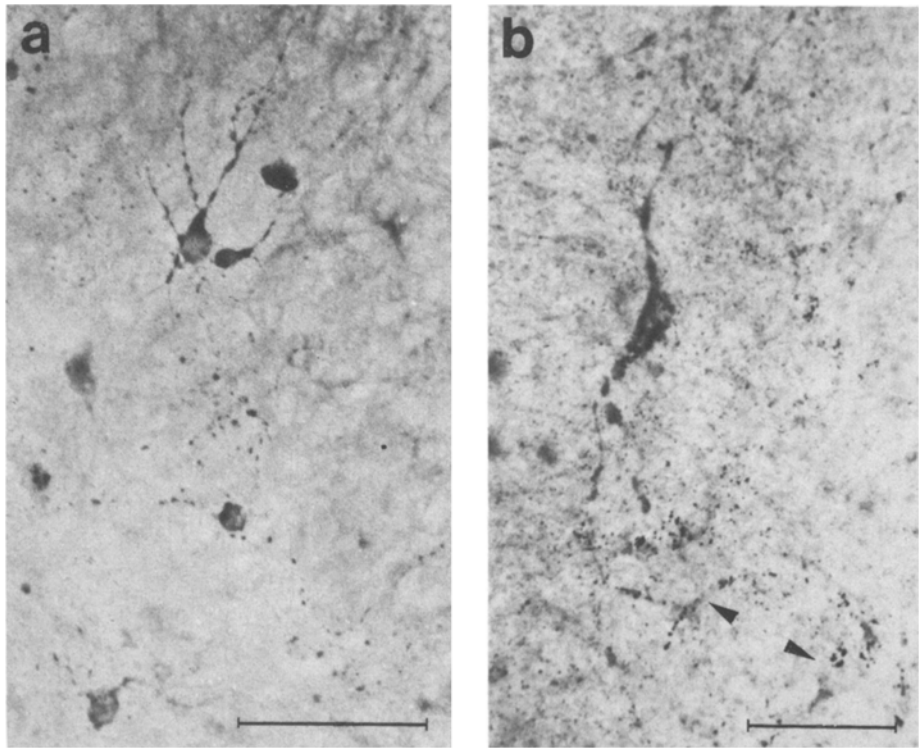

Figure 2. GABA-T cytochemistry in spinal cord cultures (21 days in vitro). *a* GABA-T-positive dorsal horn neurone and processes. *b* GABA-T-positive dorsal horn neurone showing processes which overlay unstained neurones (arrowed). Calibration bars indicate 50 µm.

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The inhibitory amino acid GABA hyperpolarizes motor axons: an intracellular study[1]

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Summary. The inhibitory amino acid γ-aminobutyric acid (GABA) hyperpolarized motor axons. This hyperpolarization was associated with an increase in the resting input conductance and with reduced action potential duration.

The putative central inhibitory transmitter γ-aminobutyric acid (GABA) has well documented actions on vertebrate primary afferent terminals[2], dorsal root ganglion cells[3,4] and motoneurones[5]. In addition extracellular recordings reveal that GABA can depolarize some peripheral axons[6] although it is not clear whether such an effect occurs on motor axons and what membrane mechanisms are associated with it.

In the course of some experiments on the frog spinal cord in vitro we encountered some units which we classified as motor axons according to the following criteria. Firstly, these cells exhibited very short latency spikes without an