Carbonic anhydrase activity in primary sensory neurons

II. Influence of environmental factors on the phenotypic expression of the enzyme in dissociated cultures of chicken dorsal root ganglion cells

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Summary. Neuronal subpopulations of dorsal root ganglion (DRG) cells in the chicken exhibit carbonic anhydrase (CA) activity. To determine whether CA activity is expressed by DRG cells maintained in in vitro cultures, dissociated DRG cells from 10-day-old chick embryos were cultured on a collagen substrate. The influence exerted by environmental factors on the enzyme expression was tested under various conditions of culture. Neuron-enriched cell cultures and mixed DRG-cell cultures (including numerous non-neuronal cells) were performed either in a defined medium or in a horse serum-supplemented medium. In all the tested conditions, subpopulations of cultured sensory neurons expressed CA activity in their cell bodies, while their neurites were rarely stained; in each case, the percentage of CA-positive neurons declined with the age of the cultures. The number and the persistence of neurons possessing CA activity as well as the intensity of the reaction were enhanced by addition of horse serum. In contrast, the expression of the neuronal CA activity was not affected by the presence of non-neuronal cells or by the rise of CO₂ concentration.

Thus, the appearance and disappearance of neuronal subpopulations expressing CA activity may be decisively influenced by factors contained in the horse serum. The loss of CA-positive neurons with time could result from a cell selection or from genetic repression. Analysis of the time curves does not support a preferential cell death of CA-positive neurons but suggests that the eventual conversion of CA-positive neurons into CA-negative neurons results from a loss of the enzyme activity. These results indicate that the phenotypic expression of cultured sensory neurons is dependent on defined environmental factors.

Key words: Dorsal root ganglion – Sensory neurons – Cell culture – Environmental factors – Carbonic anhydrase – Chicken

In the central nervous system, carbonic anhydrase (CA) is a glial enzyme that is also expressed in glial cell cultures (Delaunoy et al. 1980; Kimelberg and Ricard 1984). Cultured oligodendroglial cells are the main site of synthesis of CA II. However, a long-lasting period of culture is required to obtain detectable levels of CA. This time lag is shortened and the synthesis of CA is enhanced when the medium is supplemented with brain extracts (Delaunoy et al. 1980). Hence, glial CA may be influenced by the age of culture and environmental factors.

In the peripheral nervous system, CA is not only a glial but also a neuronal enzyme, which is located in subpopulations of dorsal root ganglion (DRG) cells and their axons (Riley et al. 1982; Wong et al. 1983; Sommer et al. 1985). In mouse DRG, the neuronal enzyme corresponds to CA II, while the CA activity detected in the satellite cells is due to another isoenzyme (Kazimierczak et al. 1986). In contrast, chicken DRG possess satellite cells that are free of CA activity (see Figs. 1, 4 and 7 of the companion paper). Turning the absence of CA in satellite cells to advantage, dissociated cell cultures of chicken embryo DRG were grown under various conditions to determine whether sensory neurons cultured in-vitro express CA activity in the absence of innervated target tissue and if so, whether various factors, e.g., age of culture, presence of non-neuronal cells, addition of horse serum or changes in CO₂ concentration, exert a role on the phenotypic expression of CA activity in cultured sensory neurons.

Materials and methods

Cell culture

Cell dissociation. DRG were dissected from 10-day-old chick embryos (Leghorn; stage 36–37) and transferred to the F14 medium (Vogel et al. 1972) at room temperature. After a short centrifugation (2 min at 500 g), the supernatant was discarded. The DRG were resuspended and incubated for 30 min in 2 ml 0.1% trypsin solution diluted in Ca⁺⁺- and Mg⁺⁺-free PBS at 37°C. After a 10-min centrifugation at 500 g, the DRG were washed with and recentrifuged in an excess of F14 medium (2 ml for 10 DRG) containing 10% (v/v) horse serum. Then, the collected DRG were dissociated in 2 ml F14 medium by trituration in a Pasteur pipette (10–15 strokes). Finally, the suspension was filtered through a nylon sieve of 48-μm mesh. The cell density of the filtrate was counted in a hemocytometer. From this cell suspension, two types of media containing 100 μl penicillin and 100 μg/ml streptomycin were prepared and two types of cell cultures were carried out.

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Three neuronal cell markers were used. Acetylcholinesterase (ACHE) activity was detected by the method of Kar- 
vsky and Roots (1964) in the presence of 10^{-4} M iso-
OMPA (tetraisopropylpyrophosphoramide) according to 
Barakat et al. (1982). Immunoreactivity for neuron-specific enolase (NSE) was shown after fixation of cultures in 4% formaldehyde in 0.2 M phosphate buffer at pH 7.5 for 1 h at 4°C, then the cultures were permeated with 0.25% Triton X-100 for 15 min prior to immunoperoxidase staining with rabbit anti-
tiserum against NSE (1:500) according to Maxwell et al. 
(1982). Controls were performed by substituting normal 
rabbit serum to D_{2}/N-CAM or NSE antiserum.

**Results**

Ovoid and refringent cell bodies, which extended elongated cytoplasmic processes, were present at each examined time interval after plating either in neuron-enriched or in mixed DRG cell cultures (Figs. 1-4). The same cells exhibited a cytochemical AChE activity or an immunocytocchemical re-
action after staining with antibodies against the neuron-
specific enolase or the D_{2}/N-CAM cell surface antigen. In 
contrast, non-neuronal cells such as fibroblasts or Schwann 
cells grown on the coating collagen were free of reaction. 
Hence, the conjugation of cytological characteristics and 
of neuronal cell markers indicated that the refringent cell 

**Cell counts.** After cytochemical detection of CA, the number of CA-positive and CA-negative ganglion cells was counted at random throughout the whole surface of the Petri dishes in order to cover an area corresponding to 1/10 of the culture.

**Cellular localization of CA activity**

In dissociated cell cultures of sensory neurons, the cytoenzy-
matic activity of CA was restricted to a fraction of the 
neuronal population and was never observed in non-neuro-
nal cells. The DRG cells possessing CA activity could not 
be morphologically distinguished from unreactive ganglion 
cells. The CA activity, which showed various degrees of 
intensity from one cell body to another, was always stronger 
in the perikaryon than in the emerging neurites (Figs. 5-7). 
In strongly reactive cells, the CoS precipitate was detected 
not only in the cytoplasm but also in the nucleus. At the 
ultrastructural level (Figs. 8-10) the CoS reaction product 
was distributed along the outer mitochondrial membrane 
and along the cytoplasmic face of the rough endoplasmic 
reticulum, frequently arranged in long and parallel cister-
nae: The density of the CoS precipitates in CA-positive 
neurons varied from one ganglion cell to another. The re-
mainder of the neurons and all the non-neuronal cells were 
free of reaction product.

**Neuron-enriched DRG cell cultures.** After 5 days of culture, the cell population consisted of 95% refringent ganglion 
cell bodies (Figs. 1, 2). Most of the neurons exhibited the spherical or ovoid shape of bipolar neurons, while a few stellate cells could be considered as multipolar neurons. The neurites appeared as long cytoplasmic expansions origi-
nating from the perikaryon. The majority of the neuronal 
cells survived for 8 days in the defined medium and for 
3 weeks in the horse serum-supplemented medium.

In the defined medium, less than 30% of the neuronal 
population displayed CA activity at 3 days; by 7 days,