Localization of Carbonic Anhydrase in the Nervous System

Abstract. Single-cell analyses of carbonic anhydrase demonstrate that this enzyme is selectively concentrated in the glial and choroid cells of the rat brain. The nerve cells show a very low carbonic anhydrase activity. This specific localization supports the view that glial carbonic anhydrase is implicated in a mechanism for the active transport of chloride from the capillaries to the interstitial and cerebrospinal fluids.

Although the presence of carbonic anhydrase in the nervous system was demonstrated as early as 1943 (*1*) and has been studied in detail as far as gross distribution of the enzyme is concerned (*2*) no conclusive data have been reported about its fine localization. This is partially due to the lack of a reliable histochemical method for the demonstration of carbonic anhydrase in tissue sections (*3*). The development of a sensitive micromethod based on a modification of the Cartesian diver technique of Linderstrøm-Lang (*4*), which allows determination of carbonic anhydrase in single tissue elements (*5*), offered the possibility not only of investigating this problem but also of providing new information on the functional relationship between glia and neurons.

Samples of single nerve cells and of the glia cells surrounding them (oligodendrocytes) were isolated under the microscope from fresh unstained preparations of the lateral vestibular nucleus of Deiters, according to the technique described elsewhere (*6*). Single-cell preparations of choroid plexus and erythrocytes were also studied. The hooded rat (Long-Evans) strain was used throughout.

Equivalent volumes of nerve and glial cells were dissected out (*6*), and their carbonic anhydrase activity was measured separately by CO_2 evolution, at 25°C, from $NaHCO_3$ (final concentration, $1 \times 10^{-4} M$) in the presence of $0.1M$ sodium phosphate buffer at *p*H 7.5. The uncatalyzed reaction and the activity curves of different known concentrations of semipurified carbonic anhydrase preparations were determined for each experiment and compared with the curve obtained with the isolated cell preparation as shown in Fig. 1.

Control experiments carried out with either the semipurified samples of carbonic anhydrase or the cell preparations in the presence of acetazoleamide ($6 \times 10^{-7} M$) showed a complete inhibition of the enzyme activity.

The enzyme activity was determined and expressed in molar terms according to the method of Maren, Parcell, and Malik (*7*).

The enzyme activity of a cell preparation which was found to be equivalent to the activity observed in a diver filled with the semipurified carbonic anhydrase solution (see Fig. 1) varied between 38 and 360 $\times 10^{-13} M$ (mean, 99 $\times 10^{-13} M$) in the glial cells, while in the corresponding nerve cells this activity was only 0.45 to 5 $\times 10^{-13} M$ (mean, 1.95 $\times 10^{-13} M$), the ratio between the glial- and the nerve-cell activity varying in a single preparation between 28 and 85 (16 experiments). Volumes varying between 18 and 50 $\times 10^{3} \mu^3$ of oligodendroglia (representing 7 to 20 glial cells) exhibited, therefore, concentrations of carbonic anhydrase (moles per unit volume) up to 120 times higher than concentrations in a single nerve cell of equivalent volume.

It is felt that the small carbonic anhydrase activity found in the nerve cells may be due to contamination with glial material.

The activity of the enzyme in a single intact red cell preparation was estimated as 2 $\times 10^{-20}$ mole; in a single nerve cell, as 3 $\times 10^{-20}$ mole; in a glial cell, as 18 $\times 10^{-20}$ mole; and in a single cell of the choroid plexus, as 300 $\times 10^{-20}$ mole.

On the basis of activity per unit volume it may be calculated that carbonic anhydrase activity in the red cell is 670 times, in the choroid cell 250 times, and in the glial cell 120 times the activity in the nerve cell of the nucleus of Deiters.

As it has been found (*8*) that the mass per unit volume of fresh glial

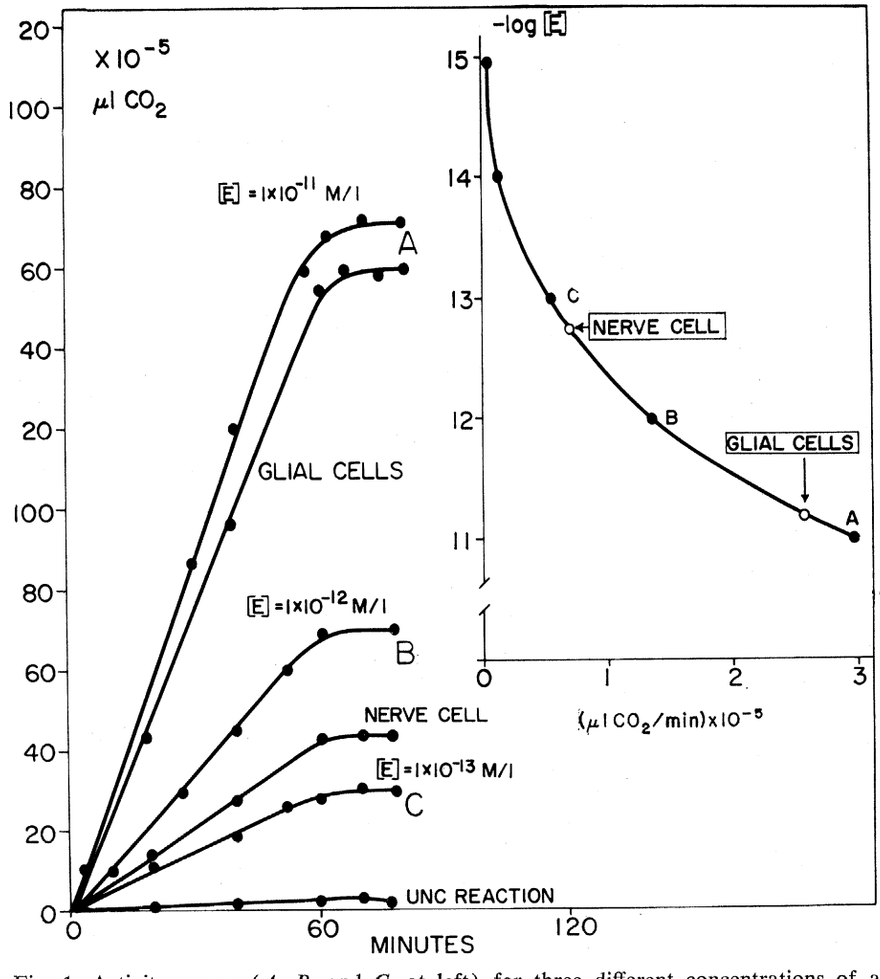

Fig. 1. Activity curves (*A*, *B*, and *C*, at left) for three different concentrations of a semipurified carbonic anhydrase preparation and the curve for the uncatalyzed (*UNC*) reaction. These are compared (insert) with the activity of a single nerve cell and an equivalent volume of glial cells.

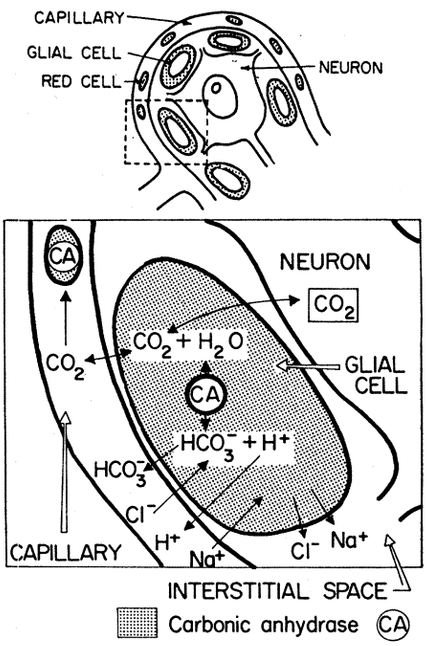

Fig. 2. (Top) The cellular localization of carbonic anhydrase in the central nervous system; (bottom) a proposed mechanism for the transport of chloride and sodium (see text).

cells in the nucleus of Deiters is the same as that of the corresponding nerve cells (about 0.20 $\mu\mu g/\mu^3$), it is appropriate to make a direct comparison of equivalent volumes of glial and nerve cells.

Studies in which the inhibition of carbonic anhydrase altered the formation and the electrolyte composition of the cerebrospinal fluid implicate this enzyme in its production (*9*, *10*). The principal change is represented by a decreased Cl^- gradient in the cerebrospinal fluid (*10*); the active transport of Cl^- is indicated by its concentration in the cerebrospinal fluid against the electrochemical gradient (*11*). The demonstration of selective high localization of the enzyme in the glial elements of the central nervous system indicates the site where this process may be presumed to act. Figure 2 shows schematically the localization of carbonic anhydrase in the nervous tissue and a possible two-step mechanism for the transport of chloride (and eventually sodium), which can be summarized as follows.

1) Carbon dioxide, which has very recently been recognized (*12*) as the immediate product of the decarboxylation reactions in the brain, can rapidly diffuse inside the neuron and, from it, into the adjacent glial cells, where it is rapidly hydrated to carbonic acid (HCO_3^- at body *p*H) in the presence of carbonic anhydrase.

2) A selective exchange of chloride from the adjacent capillary into the glial cell and from there to the interstitial space and cerebrospinal fluid can then take place.

In this way the high intracellular HCO_3^- rapidly made available from CO_2 and H_2O in the presence of carbonic anhydrase may be linked with the active transport of chloride into the interstitial space and cerebrospinal fluid.

This view represents a further extension of the concept of a secretory system (*10*) localized not only in the choroid plexus but also in the glial tissue. The very small and probably artifactitious amount of carbonic anhydrase found in the neuron may be regarded as evidence that the role of this enzyme in the central nervous system is secretory, as it is in most other sites.

It can finally be pointed out that in the structure studied (the nucleus of Deiters of the rat), the anatomical interrelationship of the glial cells to the neuron and to the capillaries (*5*) gives further support to the view that the glia has a strategic position (see Fig. 2, top) in the postulated secretion mechanism (*13*).

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Ocular Lesions Produced by an Optical Maser (Laser)

Abstract. Ocular lesions have been experimentally produced in rabbit by a pulsed optical maser (laser). The high-energy density delivered in a single 0.5 msec pulse was sufficient to cause instantaneous thermal injury to the pigmented retina and iris of the brown rabbit. Ophthalmoscopically, the retinal lesions resembled flash burns from an atomic fireball.

It is well known that the visible and near-visible regions of the electromagnetic spectrum are capable of producing thermal injury to the eye (*1*). The retina is particularly vulnerable, since the energy focused upon its surface by the refracting media is readily absorbed by the pigmented layers of the retina and neighboring choroid (*2*). Chorioretinal burns from viewing a solar eclipse or the atomic fireball are typical of the lesions that may result (*3*, *4*).

Recently, extension of molecular amplifier theory to shorter wavelengths has led to the development of optical masers, capable of generating coherent, essentially monochromatic radiation of high intensity (*5*). These devices, destined for use in communications, the medical sciences, and military installations, constitute another potential source of ocular injury due to accidental exposure.

From a consideration of the emission characteristics of a pulsed ruby maser and the transmission properties of the eye, estimates of the energy density at the retina indicate that the burn threshold may be greatly exceeded by exposing the eye to a single 0.5-msec burst (*6*). This report describes preliminary studies of retinal and iris lesions in rabbit produced by an optical maser.

A pulsed ruby maser (Vireo I laser developed by Technical Research Group) was employed. The ruby and its helical excitation source were enclosed in a cylindrical housing and mounted on an optical bench. Laser output was 0.1 joule/0.5-msec pulse, emitted in a coherent, monochromatic ($\lambda = 694.3$ mμ) beam, 1 cm in diameter. An adult, pigmented rabbit was held in a restraining box and placed on an adjustable mount with the eye approximately 30 cm from the emission face of the ruby. Pupils were maximally dilated with 2 percent Cyclogyl and 10 percent Neo-synephrine.

In rabbit, regions proximal to the optic nerve head contain medullated nerve fibers that form an elliptical area in which energy absorption is least effi-