Quinolinic acid phosphoribosyltransferase: Preferential glial localization in the rat brain visualized by immunocytochemistry

(kynurenines/excitotoxins/glia/neurodegenerative disorders)

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ABSTRACT The excitotoxic brain metabolite quinolinic acid has been hypothetically linked to the pathogenesis of neurodegenerative disorders. By using antibodies prepared against a homogeneous preparation of its catabolic enzyme, quinolinic acid phosphoribosyltransferase [QPRTase; nicotinate-nucleotide:pyrophosphate phosphoribosyltransferase (carboxylating), EC 2.4.2.19], immunocytochemical methods were applied to assess the cellular and subcellular localization of quinolinic acid in the rat brain. On the light-microscopic level, the enzyme was found to be preferentially associated with glial elements of variable morphology. In addition to its presence in glial cells, QPRTase was contained in tanyocytes and ependymal cells of the cerebral ventricles and, sporadically, in neurons. Overall, QPRTase immunoreactivity was noted in every brain region studied, the histological pattern being in good accordance with the regional variation of enzyme activity established in biochemical studies. As judged on the ultrastructural level, QPRTase, in all cell types examined so far, was often noted in densely stained roundish cytoplasmic bodies (0.1–0.8 μ m in diameter), which were bounded by a single membrane. In functional terms, these structures may represent early lysosomes, secretory granules, or residual bodies. The particular anatomical arrangement of the quinolinic acid system may reflect the brain's defense strategy against detrimental effects of the endogenous excitotoxin.

Excitotoxins, neuroexcitatory acidic amino acids with selective axon-sparing neurotoxic properties (1), have recently come to be viewed as potential pathogens in a spectrum of human neurodegenerative disorders. Based on earlier work with exogenously derived compounds such as kainic and ibotenic acids, it has been suggested that an overabundance of one or more endogenous excitotoxins in the brain may result in selective neuropathological changes (2, 3). In particular, Huntington's disease, epilepsy, and, more recently, neuronal loss associated with ischemic and hypoglycemic conditions have been hypothesized to be precipitated via excitotoxic mechanisms. Several lines of experimental evidence point to a prominent involvement in excitotoxic events of the neuronal *N*-methyl-D-aspartate receptor, a subtype of receptors for excitatory amino acids (4).

A selective endogenous *N*-methyl-D-aspartate agonist has recently been identified by electrophysiological techniques (5). The compound, quinolinic acid (Quin), has been known for decades as a hepatic intermediate of tryptophan metabolism en route to NAD⁺ (6, 7). Quin has also been shown to exist in rat and human brain tissue at a concentration of approximately 1 μ M (8, 9). Quin displays potent excitotoxic properties following intracerebral injection in rodents *in vivo* (10). In rat organotypic cultures, neuropathologic changes can be observed following exposure to as little as 10 μ M Quin

(11). All toxic effects of Quin can be prevented by selective *N*-methyl-D-aspartate antagonists, thus implicating the latter family of drugs as potential therapeutic agents in neurodegenerative diseases (12). Since Quin does not cross the blood-brain barrier under physiological conditions, the enzymatic machinery responsible for its presence in the brain was examined in cerebral tissue. Indeed, both the synthetic enzyme, 3-hydroxyanthranilic acid oxygenase (13) and the degradative enzyme, quinolinic acid phosphoribosyltransferase [QPRTase; nicotinate-nucleotide:pyrophosphate phosphoribosyltransferase (carboxylating), EC 2.4.2.19; ref. 14], exist in the rodent and human (15, 16) brain. Both enzymes, known as integral parts of the kynurenine pathway in the periphery (6, 7), are highly substrate-specific and unevenly distributed between various brain regions (13, 14). Therefore, they are uniquely qualified to yield essential information about the presence and function of Quin in the brain.

We recently purified QPRTase from rat liver and, using anti-QPRTase antibodies, demonstrated its immunological identity to the brain enzyme (17). We report here use of immunocytochemical techniques to characterize the localization of rat brain QPRTase, both at the light and electron microscopic levels, in an attempt to study the anatomical arrangements relevant for an understanding of the brain's Quin system.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Anticimex, Sollentuna, Sweden; 150–200 g) were sacrificed under deep pentobarbital anesthesia (Mebumal, ACO Läkemedel AB, Sweden; 60 mg/kg of body weight i.p.) by transcardial perfusion with 50 ml of saline (at 22°C) followed by 500 ml of a fixative prepared by either the "pH-shift" method of Berod *et al.* (18) or the method of McLean and Nakane (19). The fixed brains were cut on a freezing microtome, and 30- μ m-thick sections were incubated floating free in phosphate-buffered saline, pH 7.4/0.2% Triton X-100/1% normal goat serum containing purified rabbit anti-QPRTase antibodies (17) at a dilution of 1:6000 for 3 days. The antigen-antibody complex was made visible by the avidin-biotin-complex method of Hsu *et al.* (20) using 3',3'-diaminobenzidine (Sigma) as the chromogen. The sections either were counterstained with thionin to visualize neuronal cell bodies or were incubated in 0.2% osmium tetroxide for 3 min to enhance the 3',3'-diaminobenzidine reaction product.

Control experiments involved incubation of the sections either in nonimmune serum or in buffer containing anti-QPRTase antibodies [1 ml preabsorbed for 24 hr at 4°C with purified QPRTase (activity, 2 μ mol/hr)]. No staining occurred under either of these conditions.

Abbreviations: Quin, quinolinic acid; QPRTase, quinolinic acid phosphoribosyltransferase; IR, immunoreactive(ity).

For electron microscopic studies, rats were fixed by the "pH-shift" technique. Vibratome sections (100 μm thick) were processed by the same immunohistochemical procedure described above except that Triton X-100 was omitted and the osmium enhancement step was replaced by osmium-thiosemicarbazide-osmium treatment (21). The sections were further treated with 1% uranyl acetate in water, dehydrated in ethanol, and embedded in Epon epoxyplastic; 1- μm sections were cut parallel to the section surface. Areas with positively labeled cells were reembedded in plastic, cut in 50-nm thick sections, and examined in a Philips EM 300 transmission electron microscope.

RESULTS

Morphological Characteristics of Cells Containing QPRTase Immunoreactivity (QPRTase-IR). Specific QPRTase-IR was found by light and electron microscopy to be contained mainly in small (approximately 5- to 10- μm diameter) glial cells present in a large number of brain structures and fiber tracts throughout the neural axis of the rat brain. Moreover, QPRTase-IR also was observed in tanycytes of the mediobasal hypothalamus (Fig. 1A), in the ependymal lining of the cerebral ventricles (Fig. 1B), and in neuronal cell bodies (Fig. 1C) in several brain regions. Occasionally, a diffuse QPRTase staining was noted around major blood vessels in the basal forebrain.

In most instances, QPRTase-IR-containing glial cells had a round or oval cell body with relatively short but profusely ramifying and highly tortuous processes (e.g., Fig. 1D-F). Notably, a unipolar orientation of these processes predominated in the molecular layer of several cortical areas (e.g., Fig. 1D), including the area dentata (Fig. 1E), while QPRTase-IR glial cells had a multipolar appearance in several subcortical structures. In some brain areas, QPRTase-IR glial cells were often situated in close apposition to neuronal perikarya (Fig. 1G-J). While some of these QPRTase-IR-positive cells formed "caps" that appeared devoid of processes (Fig. 1I and J) and closely resembled the well-known satellite oligodendroglia present in cortical areas (22), other QPRTase-IR cells had one or two short processes that were in close contact with neurons (Fig. 1H). Other glial cells containing QPRTase-IR had what appeared to be numerous end-feet in close association with adjacent neurons (Fig. 1H and I). These could be seen either as granular QPRTase structures seemingly scattered over the soma surface of individual cells or dispersed in the neuropil (compare with the description of ultrastructural appearance below). QPRTase-positive glial cells located among myelinated fibers were mostly relatively small and had few processes, though larger and more elongated cells were sporadically encountered (not shown). Thus, QPRTase-IR glial cells were heterogeneous with regard to both size and morphology.

In addition to glial cells, QPRTase-IR was associated with some medium-to-large neuronal cell bodies (soma diameter, 15-20 μm). These were encountered in several brain regions but predominated in the thalamus (Fig. 1C), mediobasal hypothalamus, and striatum.

All brain areas examined so far contained QPRTase-IR glial cells. A large number of cells were present in the olfactory bulb. The neocortex and the hippocampus harbored numerous QPRTase-positive glial cells with poorly developed processes. In the hippocampus, the hilus of the area dentata was found to be especially rich in small QPRTase-IR cells, which were situated in close apposition to large neuronal cell bodies (Fig. 1J), while numerous small cells with relatively short processes were present in the Ammon's horn. QPRTase-IR cells with few, relatively sparsely branching processes were scattered throughout the brain stem, hypothalamus, caudato-putamen, and septal nuclei. Com-

pared to the cortex and the basal forebrain, the mediobasal hypothalamus was slightly less well endowed with QPRTase-IR cells of the type described above, but numerous tanycytes in this region were QPRTase-IR (e.g., Fig. 1A). Of all areas examined, the cerebellum harbored the smallest number of QPRTase-positive cells, which were found scattered between granule and Purkinje cells. Numerous QPRTase-IR glial cells were observed among myelinated fibers of all major fiber tracts, including the corpus callosum, the superior cerebellar peduncle, anterior commissure, the medial longitudinal fasciculus, the lateral olfactory tract, and the olfactory nerve.

Subcellular Localization of QPRTase-IR. In most QPRTase-positive glial cells, the reaction product appeared evenly distributed throughout the cytoplasm and nucleus. However, in some cases densely stained granules could be discerned in a less-densely stained cytoplasm. This phenomenon was particularly evident in semithin sections (Fig. 2A) and could be observed in neurons as well (Fig. 2B). By electron microscopy (Fig. 2C-F), the densely stained cytoplasmic bodies appeared roundish, bounded by a single membrane and had a diameter of 0.1-0.8 μm . The staining precipitate was too dense to allow identification of the internal structure of the granules, but they were distinct from mitochondria and Golgi vesicles. In glial cells, the less-dense cytoplasmic staining seemed to decorate most structures accessible from the cytosol in a random fashion (Fig. 2C and E). In neurons, the only QPRTase-positive structures were electron-dense cytoplasmic bodies similar to the ones observed in glial cells. Mitochondria, Golgi cisterns, and typical lysosomes with heterogeneous content were all negative (Fig. 2D and F).

DISCUSSION

By using immunohistochemical techniques, QPRTase-containing cells can be unequivocally identified in the rat brain. Overall, only a small percentage of cells can be stained with anti-QPRTase antibodies. While some QPRTase-IR is associated with neurons (see below), tanycytes, and ependymal cells of the cerebral ventricles, the Quin-catabolizing enzyme is clearly preferentially localized in glial cells. Depending mainly on the brain region, QPRTase appears to be distributed between a spectrum of morphologically distinct glial cell types. For instance, there is evidence that at least some QPRTase-positive cells in cortical areas are oligodendroglia: first, their size and limited number of cytoplasmic processes resemble that of oligodendroglia; and second, QPRTase-IR cells forming "caps" on neurons are anatomically indistinguishable from satellite oligodendroglia (22). On the other hand, numerous QPRTase-positive glial cells were found to possess several processes and are likely to represent astrocytes. Overall, QPRTase is not likely to be present in all oligodendroglial cells, and the pattern of QPRTase-IR also does not conform with a selective localization of the enzyme in all astroglia because the astrocyte-specific glial fibrillary acidic protein (23) appears to be present in other than QPRTase-IR cells in the hippocampus and cerebellum (unpublished data). The precise identification of the glial cell type(s) containing QPRTase constitutes an important task and can be approached by combined staining, on the same tissue section, with anti-QPRTase antibodies and antibodies directed against other glial markers. Thus, while the exact identity of QPRTase-IR glial cells remains to be determined and may well embrace a variety of functionally and/or morphologically distinct cells, the present study clearly demonstrates a nonrandom distribution of the enzyme responsible for the degradation of Quin in the rat brain.

In several brain areas, QPRTase-IR can be found in association with neuronal cell bodies. Light microscopic analysis suggests that in some instances the granular QPRTase-IR may represent structures located on the outer

FIG. 1. (A-J) High-power photomicrographs showing QPRTase-IR in different cell types of the rat brain. (A) QPRTase-IR in tanycytes (arrows) radiating from the wall of the third ventricle. (B) QPRTase-IR in ependymal cells of the ventricular wall. Arrows indicate granulated QPRTase-IR. Double arrowheads show nonspecific staining of endogenous peroxidase in erythrocytes. (C) QPRTase-IR granules in association with neuronal cell bodies of the laterodorsal thalamic nucleus. The exact localization (intra- vs. extracellular) remains to be established by ultrastructural studies. (D and E) QPRTase-IR glial cells (arrows) in layer I of the piriform cortex and area dentata, respectively, illustrating a unipolar orientation of the major processes. (F) QPRTase-IR glial cells with highly tortuous processes (arrows) in the striatum. (G) QPRTase-IR glial cells with distinct processes (small arrows) in close association with neuronal cell bodies (N). (H, I, and J) QPRTase-IR glial cells (arrows) in close association with neuronal cell bodies (N) in the thalamus (H), diagonal band of Broca (I), and hilus of the area dentata (J). In some cases, processes extend from the glial cells to embrace the neuron. [Bars = 20 μ m (A-C and F-J) and 10 μ m (D and E).]

FIG. 2. (A–F) High-power light micrographs of semithin plastic sections from the olfactory lobe. (A) Glial cells of the external plexiform layer reveal heavily stained cytoplasmic granules (arrows) in a less-densely stained cyto- and nucleoplasm (n) as seen by bright-field microscopy ($\times 900$.) (B) Mitral neuroperikarya with equally densely stained cytoplasmic granules (arrows) as seen by phase-contrast microscopy. ($\times 1050$.) (C–F) Transmission electron micrographs of the same material as above. (C and E) Details of QPRTase-IR glial cells. (D and F) Details of QPRTase-IR mitral cells. IR is localized mainly to cytoplasmic bodies (arrows) limited by a single membrane (double-headed arrows). Mitochondria (m), telolysosomes (tL), Golgi apparatus (G), and cisterns of the rough endoplasmic reticulum (ger) are all negative. (C, $\times 19,950$; D, $\times 7125$; E, $\times 33,250$; F, $\times 21,850$.)

soma surface of the cell, an indication of the presence of QPRTase-containing glial end-feet in direct contact with neuronal cell bodies. In addition, analysis at the subcellular level indicates that QPRTase is preferentially associated with membrane-bound roundish cytoplasmic bodies in both glial cells and neurons. While these elements can be structurally

identified as electron-dense bodies, they remain to be characterized in functional terms (e.g., as early lysosomes, secretory granules, or residual bodies).

Previous biochemical assessment of the regional distribution of QPRTase between brain areas indicates a heterogeneous localization of the enzyme (14). Of the regions studied,

the olfactory bulb, containing olfactory nerve fibers, shows by far the highest QPRTase activity. Significant but less dramatic differences are noted between other brain regions. While the intraregional variation in the density of QPRTase-IR cells and the semiquantitative nature of such a correlation must be considered, the overall picture on the regional distribution of QPRTase in the rat brain extracted from immunohistochemical analysis is in remarkably good accordance with the biochemical data obtained *in vitro* (14). Histological investigation of lesioned brain tissue in which QPRTase activity is greatly enhanced (15) should further evaluate this parallelism.

The anatomical arrangement of QPRTase-containing cells may reflect the brain's defense strategy against possibly detrimental rises in extracellular Quin concentrations. Thus, it seems noteworthy that particularly close contacts between neurons and QPRTase-IR glia exist in areas such as the cortex, the hippocampus, and the thalamus, which are known to be preferentially susceptible to the excitotoxic actions of Quin (24). Although Quin cannot be actively taken up by brain cells (25), it may, after reaching dangerously high (toxic) extracellular levels, enter QPRTase-containing cells by passive diffusion and could then be sequestered by its catabolic enzyme. Recent subcellular fractionation data (14) and the present ultrastructural analysis of QPRTase-IR are certainly in line with this hypothesis: in at least some of the immunostained glial cells (and in all of the QPRTase-positive neurons), QPRTase-IR is confined to bodies that may be envisioned to be germane for Quin degradation.

Immunocytochemical analysis of brain QPRTase can be expected to yield essential information about the physiological function and possible dysfunction of Quin in the brain.

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