Localization and Release of Homocysteic Acid, an Excitatory Sulfur-containing Amino Acid

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In addition to the excitatory role played by the amino acid transmitters glutamate and aspartate in the central nervous system, their sulfur-containing analogues homocysteic acid (HCA) and cysteine sulfinic acid (CSA) may also play a similar role. HCA is released and taken up by rat CNS tissue; it excites neurons predominantly via excitatory amino acid (EAA) receptors whenever present, and is neurotoxic. The pattern of HCA-like immunoreactivity in the rat indicates a localization of HCA mostly in glial elements, although its presence in nerve terminals and neuronal perikarya cannot be excluded. In the cerebellum of newborn and adult animals, the Bergmann glial cells and the astrocyte endfeet are immunoreactive, either in the presence or in the absence of climbing fibers. In the cortex, hippocampus, and retina, labeling is seen in both glial and neuronal elements. Excitatory signaling involving glial elements is discussed. (J Histochem Cytochem 38:1713–1715, 1990)

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Introduction

It is widely accepted that excitatory chemical neurotransmission in the vertebrate central nervous system (CNS) is mediated mostly by excitatory amino acids (EAA), particularly glutamate and possibly aspartate. Furthermore, some sulfur-containing amino acids have also been recently proposed as transmitter candidates. Specifically, the analogues of glutamate, homocysteine sulfinic acid and homocysteic acid (HCA), and of aspartate, cysteine sulfinic acid and cysteic acid, were recognized as being neuroexcitatory amino acids 30 years ago (28). Their possible importance in chemical neurotransmission was pointed out more recently when Do et al. (3) observed that they are released from depolarized rat brain slices. This review will concentrate on HCA, for which the available information suggests a neuroactive role. Release, uptake, excitatory, and cytotoxic effects, as well as immunohistochemical localization, will be considered.

Rat brain slices exposed to 50 mM K+ release HCA in a Ca**-dependent manner (5). This release was observed in all CNS regions investigated but was most prominent in cortex and hippocampus. Furthermore, in rat cerebellar slices, the small K+ -induced release of HCA is abolished when the climbing fibers are destroyed by 3-ace-tylpyridine treatment, an observation suggesting that HCA might be directly or indirectly related to climbing fibers (27).

There is evidence for the existence of an HCA uptake system (2,29; and Griffiths, personal communication). Moreover, HCA displaces the vesicular uptake of glutamate (6).

Before it was established as an endogenous releasable compound, L-HCA was shown to be a mixed excitatory agonist, acting preferentially on the NMDA receptor (3,15,16). L-HCA, when microiontophoretically applied to cat caudate neurons in vivo, induces a depolarization and firing pattern similar to that elicited by NMDA; these effects can be blocked by the selective NMDA antagonist D-2-amino-7-phosphonohexapotic acid (AP-7) (4). Moreover, L-HCA, like NMDA, induces an AP-7-sensitive [3H]-acetylcholine release from rat striatal slices (13).

In neurons of rat neocortical slices, L-HCA, but not glutamate, has been shown to preferentially activate the NMDA receptor (12, 29). Indeed, activation of the NMDA receptor by HCA does not require the adjunct action of glycine, whereas that by glutamate does (25). In cultures of mouse embryonic hippocampal neurons, HCA acts mostly as a NMDA receptor agonist (21).

In the rabbit retina, a role for L-HCA has been proposed in neurotransmission from the bipolar to the cholinergic amacrine cells.
Both light- and HCA-evoked release of acetylcholine are similarly antagonized by substances interacting with NMDA receptors (17). In the cat dorsolateral geniculate nucleus, visual stimuli and iontophoretically applied L-HCA induce similar neuronal responses, both blocked by NMDA receptor antagonists (8,9).

Finally, in cerebellar slice cultures (7) intracellular recording of Purkinje cells reveals an inward current induced by L-HCA which is fully antagonized by the non-NMDA receptor antagonist CNQX; NMDA itself, as in adult slices, had no direct effect on Purkinje cells (7,11). Furthermore, complex spikes, induced by stimulation of the inferior olive–climbing fiber system, were abolished by CNQX but unaffected by AP-5 (1).

Thus, the available evidence suggests that L-HCA excites neurons predominantly via NMDA receptors in striatum, cortex, hippocampus, retina, dorsolateral geniculate nucleus and spinal cord (4,15). In contrast, in Purkinje cells, which do not express the NMDA receptor, the excitation elicited by L-HCA appears to be mediated via a non-NMDA receptor.

**Neurotoxicity**

High concentrations of EAA induce in the CNS specific patterns of cell damage mediated via their receptors. L-HCA neurotoxicity in cortical neurons is blocked by the selective NMDA antagonist DL-2-amino-5-phosphonovalerate (AP-5) (10) and in chick retina by AP-7 (18). In addition, the morphological pattern of cell degeneration produced by L-HCA in chick retina is more similar to that obtained with NMDA than with Glu or kainate (18,22).

**Immunohistochemical Localization**

HCA has been immunohistochemically localized mainly in cerebellum, but also in cortex and hippocampus of the rat by Streit and collaborators (24) and in the retina by Grandes and collaborators. To localize homocysteate-like immunoreactivity, a sensitive post-embedding staining procedure for semi-thin sections from animals rapidly perfusion-fixed with aldehydes (14) was applied, using polyclonal mouse antibodies raised against glutaraldehyde-linked HCA-albumin conjugates. In a test system introduced by Ottersen (20), immunoreactivity was found with these antibodies only for a glutaraldehyde-linked amino acid–protein conjugate containing HCA among 25 conjugates assayed.

The patterns of HCA-LI at the light microscopic level are compatible with a localization mostly in glial elements, although the presence of HCA in nerve terminals and neuronal perikarya cannot be excluded. In the rat cerebellum, HCA-like immunoreactivity is found in the molecular layer, most frequently as fibrous elements having a radial orientation, although variocities can also be observed in close association with large Purkinje cell dendrites. Large immunoreactive dots surround the Purkinje cell perikarya. HCA-immunoreactive dots of various sizes are also present in the surrounding blood vessels, in the granule cell layer, and in the white matter. This pattern suggests the presence of immunoreactive material in parts of Bergmann glial cells and in astrocyte endfeet. The use of alternating ultra-thin and semi-thin sections, the former for electron microscopy and the latter for immunostaining, enables definitive identification of elements containing the immunoreactivity as glial in nature. Indeed, in the molecular layer, such varicose profiles associated with large Purkinje cell dendrites can be defined as part of their surrounding glial sheath. Furthermore, the radial fibrillose immunoreactive elements show the characteristics of Bergman glia. The cerebellar pattern of HCA-like immunoreactivity appears to be fairly specific, as it is very different from that observed with antibodies that recognize glutamate or GABA.

In cerebellum of newborn rat, the pattern of HCA-like immunoreactivity is similar to that observed in adults, reinforcing the interpretation that the antigenicity is localized primarily in Bergmann glia rather than in climbing fibers, as the latter have not yet reached the molecular layer by that time. Furthermore, in 3-AP-treated rats, HCA-like immunoreactivity does not decrease noticeably despite complete destruction of the inferior olivary neurons, an observation which again makes it unlikely that HCA is primarily localized in their climbing fibers. Finally, in the pigeon, the same pattern as in the rat is observed, but the HCA-like immunoreactivity of the cell bodies of Bergmann glia is more evident.

In the hippocampal formation, HCA-immunoreactive dots are dense in the infragranular zone of the dentate gyrus, numerous between the the cell bodies of the dentate granule layer and of the CA3 pyramidal cell layer, and frequent in the dentate molecular layer, surrounding blood vessels, and in the outer part of the CA1 stratum radiatum. This pattern seems to correspond to labeling of certain astrocyte endfeet. Very rarely, however, HCA-like immunoreactivity can also be identified in neuronal perikarya of the CA1 and CA3 regions.

In the cerebral cortex, preliminary observations suggest that the astrocyte endfeet are HCA immunoreactive. Relatively large and intensely immunoreactive puncta are grouped in the subcortical white matter.

In the rat retina, the following pattern of HCA-like immunoreactivity has been observed by Ortega et al. (19). In the inner nuclear layer, some cell bodies are intensely reactive, while many others display moderate cytoplasmic staining. Immunoreactivity is also observed in the outer and inner plexiform layers; stained dots are numerous in the latter, but are more densely accumulated in a band within the external part of this layer. In the ganglion cell layer, large HCA-immunoreactive dots can be seen between and below the ganglion cell perikarya. Some dots are seen in close apposition to blood vessels of the inner plexiform and ganglion cell layers. The immunoreactive elements, whether glial, neuronal, or both, cannot be defined at present.

**Discussion**

Although the information reviewed above concerning release, uptake, and neuroactivity of HCA would be compatible with an excitatory neurotransmitter role, similar to that assumed for glutamate or aspartate, the localization of the HCA-like immunoreactivity predominantly in glial elements raises a problem. At least two interpretations can be proposed (24). (a) Under physiological conditions, HCA would be present in nerve terminals, where it would operate as a classical transmitter; during perfusion, it would leak massively from the terminals and would then be taken up by glial cells, where its immunoreactivity would be detected while that left in terminals would not. (b) HCA would be primarily localized in...
glia and released from this compartment when the glial cells are depolarized, possibly by an excitatory transmitter, it would then activate extrasynaptic receptors. The data available for the cerebellum tend to favor the second hypothesis because in this location, after degeneration of climbing fibers, the Ca++-dependent HCA release in vitro is abolished while the glial immunoreactivity is normal. Kainate binding proteins, recently shown to be present on the surface of the Bergmann glia cells of lower vertebrates (23), could play the role of receptors and mediate the effect induced by the climbing fiber transmitter. Moreover, functional EAA receptors have been demonstrated in rat cerebellar astrocytes (26). Nevertheless, more decisive data are required to firmly establish one of the two hypotheses, especially for the second, which implies a completely new mechanism of signal transmission.

Literature Cited

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