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Release of neuroactive substances: homocysteic acid as an endogenous agonist of the NMDA receptor

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Summary. Sulfur containing amino acids such as homocysteic acid (HCA), cysteinsulfinic acid, homocysteinsulfinic acid are released by depolarization of slices from various rat brain regions in a Ca^{++}-dependent manner. L-HCA excites caudate neurons through their N-methyl-D-aspartic acid (NMDA) receptor and potentiates their cortically evoked excitatory postsynaptic potentials. ^{35}S-methionine can label the releasable pool of HCA, and thus appears as a precursor of HCA. Thus HCA is a transmitter candidate which acts predominantly on the NMDA receptor.

Keywords: Homocysteic acid, NMDA receptor, excitatory amino acid, release.

Introduction

The acidic amino acids are potent excitants in the central nervous system and as such have been implied in the generation of seizure activity, together with their structural and functional analogues (Cuénod and Streit, 1983; Croucher et al., 1982; Fonnum et al., 1981; French et al., 1982; Meldrum, 1985; Streit, 1984; Watkins and Evans, 1981).

Material and methods

Recently, Do et al. (1986a) investigated the release induced by 50 mM $[K^+]$ in rat brain slices. The superfusates were analysed by reversed-phase HPLC using a precolumn derivatization with 4-N,N-dimethylamino-azobenzene-4′-isothiocyanate (DABITC), as modified from the method described by Chang (1981). This method allows to detect compounds having a free amine group and, in the case of peptides, to determine their amino acid composition and N-terminus sequence.

Results and discussion

The presence and release of endogenous sulfur containing amino acids was demonstrated in various rat brain regions: homocysteic acid (HCA) in all regions but particularly in neocortex and hippocampus, cysteine sulfinic acid (CSA)

and homocysteine sulfinic acid (HCSA) in neocortex, hippocampus, mesodiencephalon and, for HCSA, in striatum. The concentration of HCA collected from cortical slices ranged from less than 0.08 ± 0.01 pmole/mg protein/min of superfusion (under resting condition) to 0.59 ± 0.13 pmole/mg/min (under stimulation condition). Thus, the relative release of HCA in the cortex amounted to 7.3 and was in all structures the most prominent one among sulfur containing amino acids. The release was Ca^{++}-dependent and could also be induced by veratrine (33 μg/ml) (Do et al., 1986a).

HCA, CSA, and HCSA have been reported by Curtis and Watkins (1960) and Mewett et al. (1983) among others to exert an excitatory effect on CNS neurons, HCA being 12 times more potent than glutamate. Their release upon depolarization as endogenous compounds supports the hypothesis that they might play a role as neurotransmitters. While in the case of CSA, such a hypothesis is well founded (Recasens et al., 1984; Iwata et al., 1982), less information is available for HCA. It is, however, taken up with high and low affinities (Cox et al., 1977) and its effect can be blocked by selective antagonists of the N-methyl-D-aspartic acid (NMDA) receptor (Mewett et al., 1983; Baudry et al., 1983; Luini et al., 1984).

Herrling and Turski (1986) and Do et al. (1986b) tested the effect of microiontophoretically applied L-HCA on intracellularly recorded cat caudate neurons (Herrling et al., 1983). The long lasting depolarization and burst of action potentials induced by L-HCA was very similar to that elicited by NMDA and both were blocked by 2-amino-7-phosphonoheptanoic acid (AP-7), a selective and potent NMDA antagonist (Evans et al., 1982; Perkins et al., 1982). In contrast, quisqualic acid or kainic acid application induced a regularly spaced short depolarization with few action potentials practically unaffected by AP-7. Similarly, in the frog spinal cord in vitro, the depolarization induced by L-HCA or NMDA was strongly antagonized by AP-7. Furthermore, it was shown that the cortically evoked excitatory postsynaptic potentials recorded in caudate neurons were enhanced by microiontophoretic application of L-HCA.

Thus, L-HCA is an endogenous excitatory amino acid which is released upon depolarization in vitro and seems to affect predominantly the NMDA receptor. It, therefore, fulfills many criteria required for a neurotransmitter (Cuénod et al., 1986). It is interesting to note that, in pyramidal cells of rat cortical slices, Thomson et al. (1985) observed synaptic potentials, evoked by stimulation of the white matter, which could be characterized as originating from an NMDA receptor-mediated synapse. In view of the results reported above, HCA, whose cortical release was particularly prominent, could be involved at synapses of this type. Alternatively, quinolinic acid, which is present in rat brain (Wolfensberger et al., 1983) and is an excitotoxin (Schwarcz, 1983, 1984) acting as an NMDA receptor preferring agonist (Stone, 1985) could be a transmitter candidate at such sites; however, no evidence has been presented to date indicating that quinolinic acid is released from nervous tissue during depolarization.

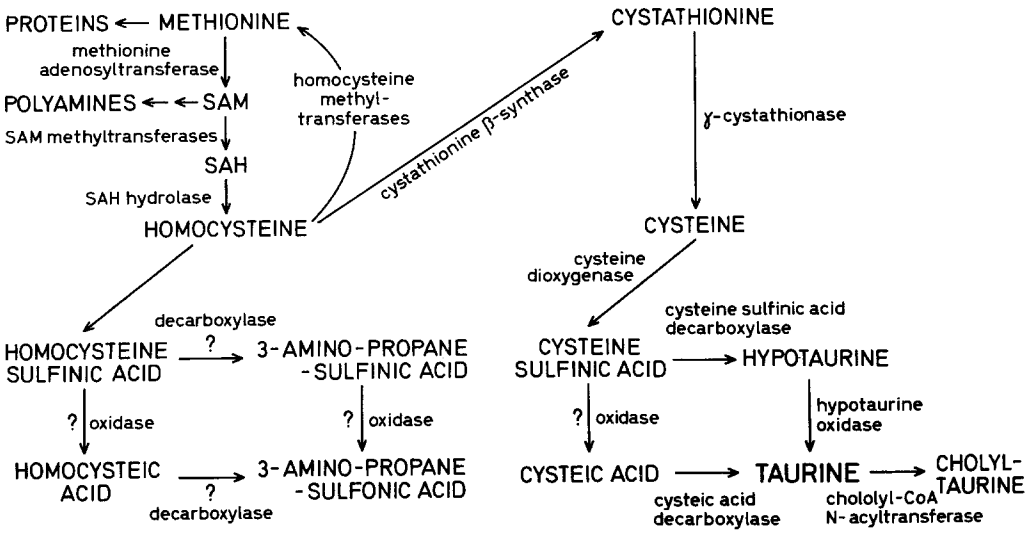

Fig. 1. Methionine transsulfuration metabolic pathways: The biosynthesis of homocysteic acid and 3-amino-propane-sulfonic acid from homocysteine (bottom left) is proposed by analogy with the established pathway from cysteine to cysteic acid and taurine (bottom right)

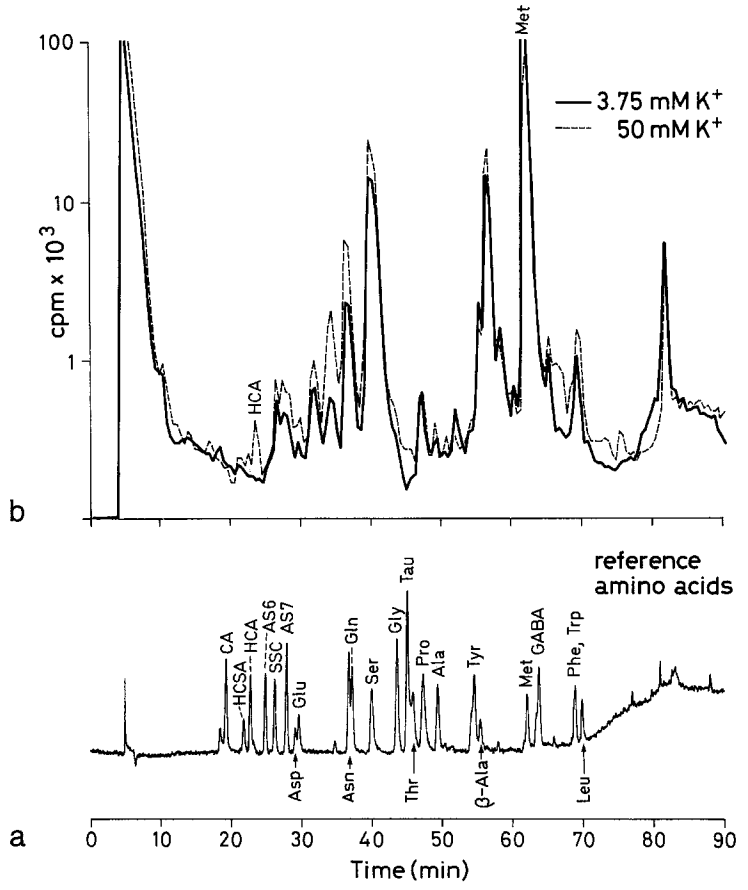

Fig. 2. HPLC analysis of radioactive substances released upon K^+-depolarization from rat cortex slices preincubated with ^{35}S-Met. **a** Chromatogram of a mixture of reference amino acids-DABTC derivatives. **b** Radioactivity in 0.5 min fractions collected from HPLC analysis of DABITC derivatized superfusates. Comparison between resting (3.75 mM K^+) and stimulation (50 mM K^+) conditions show the release, among others, of a ^{35}S-labeled peak eluting at the same retention time as HCA

In order to characterize the biosynthesis of HCA and possibly to increase the sensitivity of its detection, attempts were made to label it from radioactive precursors. As can be seen in Fig. 1, the metabolism of methionine to cysteine sulfinic acid, cysteic acid and taurine through cysteine is relatively well established, while a biosynthetic pathway from homocysteine to HCA is proposed here by analogy. To test this hypothesis, rat cortical slices were preincubated with ^{35}S-Met and then, as described above, superfused with solutions containing 3.75 mM or 50 mM [K^+]. The superfusates were derivatized and analysed by HPLC and the radioactivity determined by scintillation counting in each fraction. Several peaks of ^{35}S-labeled compounds were observed, some of them clearly increased during depolarization (Fig. 2). In particular, at the retention time of HCA, a peak of radioactivity was detected only under depolarizing conditions, suggesting that the HCA released was ^{35}S-labeled. This observation supports the idea that Met is a precursor of HCA and opens new ways of investigating this transmitter candidate.

In this context it is worth mentioning that HCA has been reported to be present in excess in urine of homocystinuric patients which also suffer from epileptic seizures (Mudd and Levy, 1983; Ohmori et al., 1972). In these patients a deficiency of cystathionine-β-synthase leads to an increased level of homocysteine in plasma and brain tissue (Sprince et al., 1969).

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