Neurochemical Characterization of Cysteine Sulfinic Acid, an Excitatory Amino Acid, in Hippocampus

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Abstract—In this communication, I have summarized our studies on the possible roles of cysteine sulfinic acid (CSA) in the central nervous system (CNS). From these observations, CSA was suggested to be a neurotransmitter. We reported the presence of CSA in the CNS and subsequently characterized Na⁺-dependent high affinity uptake and depolarization-induced release of CSA. Depolarization-induced release of [¹⁴C]CSA from the preloaded hippocampal slices was specifically attenuated by benzodiazepines and GABA agonists. Synaptic membranes have a Na⁺-independent specific binding site for cysteic acid, an analogue of CSA, which may be a possible binding site for CSA. This binding site seemed to be distinct from that for glutamate. To assess CSA as a physiologically active candidate which is distinct from glutamate, two neurochemical experiments were performed: one experiment determined the enhancement by excitatory amino acids of depolarization-induced release of [³H]GABA from the preloaded slices, and the other one monitored the cyclic AMP formation by excitatory amino acids in hippocampal slices. In both studies, differences in the responses to the various antagonists indicate that CSA receptors are distinct from glutamate receptors. Furthermore, we proposed that excitatory amino acid receptors which are subsequently linked to adenylate cyclase are functionally related to the Cl⁻ channel.
briefly describe some neurochemical characterizations of CSA in the CNS, especially in the hippocampus, which were mainly performed in our laboratory.

1. Metabolism, uptake and release

CSA is synthesized from cysteine by cysteine oxidase and is converted to taurine by CSA decarboxylase. These enzymes are localized in synaptosomes (13). Synaptosomes can accumulate extracellular cysteine and synthesized CSA and taurine (14, 15). Although the electrophysiological study had revealed the excitatory effects of CSA in the central neurones (4), there had been no information on this amino acid. In 1980, we used an enzymatic cycling method and reported the presence and the distribution of CSA in rat CNS (16). Later, HPLC methods were developed for the determination of CSA in brain preparations (17, 18). CSA is distributed unevenly in rat brain, but its content is much lower than that of glutamate. Since oxidation of CSA to CA occurs non-enzymatically, it is difficult to estimate the precise concentration of CSA. CSA is a substrate for both CSA decarboxylase and CSA transaminase. The former enzyme initiates a pathway leading to taurine; the latter enzyme forms $\beta$-sulfinylpyruvate, which spontaneously decomposes to pyruvate (Fig. 1). The specific activity of CSA transaminase is much higher than that of CSA decarboxylase in rat cerebral cortex and striatum, showing that the pathway leading to pyruvate is likely to be a major route for CSA metabolism in the CNS. Detailed characterization of the enzymes in CSA metabolism have been reviewed elsewhere (19). It should be noted that the characterization of cysteine oxidase of the brain remains to be clarified. We examined the uptake and release of CSA by synaptosomes and slices of rat cerebral cortex (20). Like many other substances, CSA is accumulated in synaptosomes by a Na-dependent high affinity uptake system of which the $K_m$ value is 12 $\mu$M. None of the centrally acting agents had any effect on the uptake of CSA at the concentration of less than 10 $\mu$M. Glutamate, aspartate and cysteic acid are competitive inhibitors for the uptake of CSA, indicating that CSA may be transported into synaptosomes by the high affinity carrier system for acidic amino acids (20).

One of the important criteria for neurotransmitters is that the candidates are released by depolarization in a Ca$^{2+}$-dependent manner. We, therefore, next examined the release of [14C] CSA from the preloaded slices and synaptosomal fractions by a superfusion method (20). Like other putative transmitter substances, CSA is released from
the preloaded preparations in a partly Ca\(^{2+}\)-
dependent manner by depolarization. Recently, Do et al. (18) reported that
depolarization caused in vitro release of endogenous excitatory sulfur-containing
amino acids including CSA from slices of various rat brain regions.

Inhibition of CSA release by benzodiazepines (21): When the pharmacological dose
of CSA is injected intraventricularly, it causes strong EEG seizures which initiate in the
hippocampus and propagate to the cortex (7). Therefore, in the following experiments,
the preparation used was the hippocampus. Benzodiazepines and meprobamate, but not
chlorpromazine, diphenylhydantoin and hexobarbital, at a concentration of 100 \(\mu\)M
significantly inhibited the depolarization-induced release of \([^{14}C]\) CSA from the
preloaded rat hippocampal slices without affecting the spontaneous release. The
inhibition by diazepam is relatively specific, because it also inhibited the release of
glutamate without affecting the release of GABA, ACh, noradrenaline and dopamine.
IC\(_{50}\) values of diazepam for high K\(^+-\)induced release of CSA and glutamate were about
20 \(\mu\)M and 7 \(\mu\)M, respectively. GABA is an inhibitory neurotransmitter of basket cells
which innervate granule cells, excitatory amino acid neurons, in the hippocampus
(22). As did the benzodiazepines, GABA and mscimol at the concentration of 100 \(\mu\)M
also reduced the release of CSA. Bicuculline, a GABA antagonist, which by itself had no
significant effect, antagonized the inhibitory effects of diazepam and GABA. Similar
results were obtained with the glutamate release. These results indicate the modulation
by GABA innervation of the release of excitatory amino acids in rat hippocampal
formation, and they also suggest that some of the pharmacological effects of diazepam
may be a consequence of inhibition of excitatory amino acid transmission.

2. Binding site for CSA (23, 24)

Among the excitatory amino acids, the Na\(^+\)-independent specific binding of gluta-
mate to synaptic membranes has been studied extensively (for review, see refs. 2
and 4). It is, therefore, of interest to examine whether synaptic membranes have a specific
binding site for CSA that differs from glutamate binding sites. We used \([^{35}S]\)-
cysteic acid (CA), an analogue of CSA, as a ligand for the binding assay (23). Scatchard
analysis of Na\(^+\)-independent specific binding of CA indicated a single population of binding
sites, with a \(K_d\) of 474 nM and a \(B_{max}\) of 3.29 pmol/mg protein. Displacement studies with various structural analogues showed that among the excitatory amino acids
tested, L-CSA was the most effective displacer, following by L-glutamate, L-CA
and L-aspartate. The \(K_i\) value of L-CSA was about 1/14 of that of L-glutamate. Two
conclusions can be derived from the observations described above. First, synaptic
membranes have a Na\(^+\)-independent specific binding site for CA, which may be a possible
binding site for CSA. Second, this binding site seems to differ from that for glutamate.

Recasens et al. (25, 26) used \([^{3}H]\) CSA as a ligand and also indicated the presence of distinct binding sites for CSA and glutamate
in synaptic membranes. In addition, our recent study showed that the binding site
for CSA has a complete dependency on Cl\(^-\), showing that the characteristics of CSA
binding are not identical to glutamate binding (Baba et al., in preparation). Thus,
the radioligand binding assay indicates the presence of a specific binding site for CSA in
synaptic membranes. However, to assess CSA as a physiologically active candidate
which is distinct from glutamate, it is necessary to find some specificities in the
actions of CSA. This was investigated in the following two neurochemical experiments.

3. Neurochemical effects

Potentiation of GABA release: Glutamate
and similar substances are likely to be the
neurotransmitters of several afferent and
efferent pathways, and GABA seems to be
a neurotransmitter for basket cell inhibition
of pyramidal and granule cells in the hip-
cocampal formation (22). To differentiate
CSA neurones, if any, from glutamate
neurones, we characterized the stimulation of
GABA release by excitatory amino acids
especially CSA in rat hippocampal slices
(27). Excitatory amino acid agonists, such as
CSA, glutamate, kainate (KA) and quis-
qualate (QA), significantly potentiated the
high K+ (25 mM)-induced release of [3H]-GABA from the preloaded hippocampal slices at concentrations above 0.1 mM. Among these agonists, CSA was the most potent. N-methyl-DL-aspartate (NMA), another agonist, did not enhance the release. In contrast, CSA had no significant effect on the high K+-induced release of ACh. In addition, the stimulatory effect of CSA on the release of GABA was negated by tetrodotoxin and was not observed in the crude synaptosomal fractions of the hippocampus. These results indicate the neuronal circuit is absolutely required for the stimulatory effect of CSA.

In view of the electrophysiologic studies, NMA, KA and QA are selective agonists to three different types of receptors for excitatory amino acids (3), which exhibit differential sensitivities to blockage by various antagonists. Table 1 summarizes the effects of various excitatory amino acid antagonists on CSA- and glutamate-induced release of GABA from the preloaded slices (ref. 27 and A. Baba et al., in preparation). It is clear that the stimulatory effects of CSA and glutamate have different sensitivity to the antagonists. Thus, CSA receptors involved in the enhancement of GABA release seems to be distinct from glutamate receptors. However, since these antagonists are relatively nonspecific, examinations must be carried out using more specific antagonists for a further evaluation of the receptors for CSA.

Enhancement of cyclic AMP (cAMP) formation: Excitatory amino acids markedly increased the formation of cAMP in incubated brain slices (28, 29). The mechanisms by which excitatory amino acids elevate the cAMP level in brain slices were believed to be common, i.e., common receptors or release of adenosine (28–30). However, our studies on the mechanisms of CSA-induced formation of cAMP in guinea pig hippocampal slices (31–34) conflict with this hypothesis. CSA (0.1–10 mM) greatly increased the cAMP level in hippocampal slices. CSA was more potent than glutamate and aspartate, and its effect was selectively antagonized by 0.1 to 30 mM taurine. This inhibition by taurine was observed in hippocampal slices but not in cortical slices (31). To identify the receptors that are involved in the stimulatory effect of CSA and in the inhibitory effect of taurine on cAMP formation, various agonists and antagonists of excitatory amino acids were examined for their possible effect on cAMP formation (32). Excitatory amino acid agonists such as NMA, KA and QA stimulated the formation of cAMP in the slices. Effects of these agonists were more pronounced in the hippocampus than in the cerebral cortex. NMA was less effective than KA and QA. Taurine markedly reduced the stimulatory effects of KA and QA without affecting that of NMA. With respect to the effects of the antagonists, whereas D-α-aminoadipate itself showed an agonist-like effect, CSA-induced formation of cAMP was significantly antagonized by D-α-aminoadipate and also by glutamate diethylster. These antagonists had no effect on the stimulation by glutamate and aspartate (32). These studies clearly demonstrate the heterogeneity of excitatory amino acid receptors that are involved in the formation of cAMP.

Unlike other transmitters, it is not likely that excitatory amino acid receptors are directly coupled with adenylate cyclase. In fact, neither of the excitatory amino acids activate adenylate cyclase activity in a cell-free system from the hippocampus under any conditions. As has been suggested (35, 36), the stimulated formation of cAMP by

| Compound | DAA | GDEE | K.A. | S.B. | Mg²⁺ | APB | Zn²⁺ | Insulin |
|----------|-----|------|------|------|------|-----|------|--------|
| CSA      | –   | –    | +    | +    | –    | +   | +    | +      |
| Glutamate| +   | –    | –    | +    | ND   | –   | –    | –      |

Antagonism (+), No antagonism (–), Not determined (ND). DAA: D-α-aminoadipate, GDEE: Glutamate diethylster, K.A.: Kynurenic acid, S.B.: Secobarbital, APB: 2-Amino-4-phosphonobutyric acid.
CSA was also inhibited by adenosine deaminase and 2'-deoxyadenosine. Thus, activation of the receptors by the agonist might be accompanied by a subsequent release of adenosine. Other approaches were made to evaluate excitatory amino acid receptors which were involved in the formation of cAMP (33, 34); the treatment of slices with polyunsaturated fatty acid resulted an enhancement of the functional coupling between adenylyl cyclase and norepinephrine- and and adenosine-receptors. In contrast, the treatment had no significant effect on the response to CSA and glutamate. This indicates that the coupling between excitatory amino acid receptors and adenyl cyclase is qualitatively different from those of other neurotransmitters. Our recent study further provides new aspects of excitatory amino acid-induced formation of cAMP in hippocampal slices (Baba et al., in preparation). Forskolin, an activator of adenyl cyclase, has two distinct effects on the adenyl cyclase system. It markedly activates the catalytic unit of the enzyme at relatively high concentrations and enhanced the responses to hormones at relatively low concentrations (37). In our study, forskolin markedly enhanced the responses to histamine and adenosine, but drastically attenuated the stimulation by CSA. Furthermore, recently we have indicated the relation between excitatory amino acid-induced cAMP formation and the Cl" channel. Many studies showed the presence of Cl"-dependent binding of glutamate in synaptic membranes (38), although its correlation to the biological effects of glutamate is not clear. In our cAMP studies, when the Cl" was omitted from the medium, the stimulation of cAMP formation by CSA and glutamate were completely negated. The anion selectivity(258,868),(762,888) for the stimulation by CSA was in parallel to that for the Cl" channel. Thus, the enhancement of cAMP formation by excitatory amino acids absolutely required the presence of Cl". Results of these studies are summarized in Table 2.

### Table 2. Summary of the effects of various compounds on the CSA-induced formation of cyclic AMP in hippocampal slices

| Agonist     | Agonist activity | Taurine | DAA | GDEE | FK | LA | Cl"-free |
|-------------|------------------|---------|-----|------|----|----|----------|
| CSA         | highest          | –       | –   | –    | –  | ±  | –        |
| Glutamate   | high             | ±       | –   | –    | –  | ±  | ND       |
| Norepinephrine | low            | –       | ND  | ND   | +  | +  | –        |
| Adenosine   | low              | –       | ND  | ND   | +  | +  | –        |

Potentiation (+), Suppression (–), No change (±), Not determined (ND). DAA: D-α-amino adipate, GDEE: Glutamate diethylester, FK: Forskolin. LA: Linoleic acid.

4. Conclusion

In the present communication, several neurochemical characteristics of CSA were described. These characteristics were common to those observed for many other neurotransmitter candidates. Furthermore, the present communication indicates that the neurochemical characteristics of CSA seem to be distinct from those of glutamate. Recently, similar lines of evidence were reported by others (18, 25, 26, 39). At present, the biochemical and pharmacological qualifications of “CSA neurones” are not clear. However, on the basis of the observations described above, it is likely that CSA can serve as an excitatory neurotransmitter in the CNS.

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