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Excitatory Amino Acids: Studies on the Biochemical and Chemical Stability of Ibotenic Acid and Related Compounds

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Abstract: The complex pharmacological profile (excitation/inhibition) of ibotenic acid on single neurons in the mammalian CNS prompted studies on the stability of ibotenic acid and a number of structurally related excitatory amino acids under different in vitro conditions in the presence or absence of enzymes. Ibotenic acid, (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid (7-HPCA), (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and (*RS*)-α-amino-3-hydroxy-4-bromo-5-isoxazolepropionic acid (4-Br-homoibotenic acid) were all inhibitors of (*S*)-glutamic acid decarboxylase (GAD) in mouse brain homogenates, but only ibotenic acid was shown to undergo decarboxylation during incubation with brain homogenates. The formation of the decarboxylated product, muscimol, which primarily occurred in a synaptosomal fraction, was dependent on the presence of pyridoxal-5-phosphate (PALP) and was inhibited by (*S*)-glutamic acid, 3-mercaptopropionic acid (3MPA), aminooxyacetic acid (AOAA), and allylglycine, suggesting that ibotenic acid is a substrate for GAD. The overall decomposition rate for ibotenic acid (8.7 nmol min^{-1} mg^{-1} of protein), which apparently embraces other reactions in addition to decarboxylation to muscimol, was higher than the rate of decarboxylation of (*S*)-glutamic acid (3.2 nmol min^{-1} mg^{-1} of protein). At pH 7.4 and 37°C, but in the absence of enzymes, none of the excitatory amino acids under study underwent any detectable decomposition, whereas ibotenic acid and 7-HPCA, but not AMPA and 4-Br-homoibotenic acid, decomposed, partially by decarboxylation, at 100°C in a pH-dependent manner. In the presence of liver homogenates, ibotenic acid was also shown to decompose. Although muscimol was the only detectable reaction product, mechanisms other than decarboxylation may be involved. Under these conditions, the degradation reaction or reactions were partially dependent on PALP and were inhibited by AOAA and 3MPA but not by allylglycine. The present in vitro studies indicate that ibotenic acid is likely to undergo enzyme-catalyzed decomposition to give muscimol in brain tissue and after systemic administration to animals. These aspects must be taken into consideration in the interpretation of the pharmacological or neurotoxic effects of ibotenic acid after direct application near central neurons, after local injections into animal brains, or after systemic administration to animals. **Key Words:** Ibotenic acid—*N*-Methyl-(*R*)-aspartic acid agonist—Glutamic acid—(*S*)-Glutamic acid decarboxylase—Excitation—Stability—Neurotransmission—Muscimol. **Nielsen E. Ø. et al.** Excitatory amino acids: Studies on the biochemical and chemical stability of ibotenic acid and related compounds. *J. Neurochem.* **45**, 725–731 (1985).

Ibotenic acid (Fig. 1), which is biosynthesized by the mushroom *Amanita muscaria* (Eugster, 1969; Lund, 1979), is a conformationally restricted analogue of (*S*)-glutamic acid. Although ibotenic acid quite effectively inhibits the receptor binding of (*S*)-glutamic acid (Foster and Roberts, 1978), aspartic acid (Sharif and Roberts, 1981), and kainic acid (Simon et al., 1976), its potent neuroexcitatory ac-

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Abbreviations used: AEMI, 5-methyl-4-(2-aminoethyl)-3-isoxazolol; AET, 2-aminoethylisothiouronium bromide hydrobromide; AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AOAA, aminooxyacetic acid; 4-Br-homoibotenic acid, (*RS*)-α-amino-3-hydroxy-4-bromo-5-isoxazolepropionic acid; 4-Br-homomuscimol, 4-bromo-5-(2-aminoethyl)-3-isoxazolol; GAD, (*S*)-glutamic acid decarboxylase; 7-HPCA, (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid; 4-methylibotenic acid, (*RS*)-α-amino-3-hydroxy-4-methyl-5-isoxazoleacetic acid; 4-methylmuscimol, 4-methyl-5-aminomethyl-3-isoxazolol; 3MPA, 3-mercaptopropionic acid; NMA, *N*-methyl-(*R*)-aspartic acid (*N*-methyl-D-aspartic acid); PALP, pyridoxal-5-phosphate; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol.

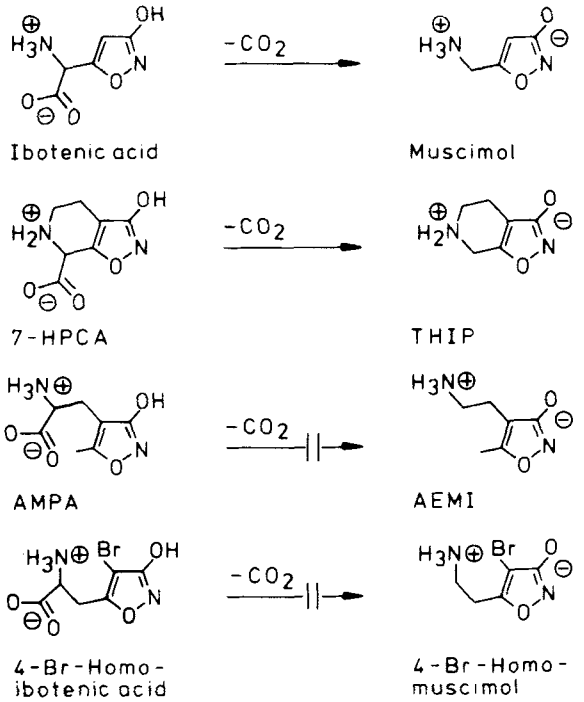

FIG. 1. The structures of ibotenic acid and a number of ibotenic acid analogues and the corresponding decarboxylated amino acids.

tions (Johnson et al., 1968; MacDonald and Nistri, 1978; Curtis et al., 1979; Krogsgaard-Larsen et al., 1980) are mediated primarily by the *N*-methyl-(*R*)-aspartic acid (NMA)-preferring receptors (Lambert et al., 1980; Watkins, 1981; Watkins and Evans, 1981; Engberg et al., 1983). In addition to these complex structure-activity relations (Krogsgaard-Larsen et al., 1984*b*), electrophysiological studies have disclosed an unusual pharmacological profile of ibotenic acid. MacDonald and Nistri (1978) observed a biphasic response to ibotenic acid of spinal neurons in barbiturate-anesthetized cats, consisting of an initial excitation followed by a prolonged bicuculline- and strychnine-insensitive depressant action, making a majority of the neurons tested progressively less sensitive to excitation by ibotenic acid or other excitants. The biphasic effect of ibotenic acid was confirmed by Curtis et al. (1979), and in addition these authors found the prolonged depressant phase of the action of ibotenic acid virtually identical with the effect of muscimol in terms of time course, reversibility, and sensitivity to bicuculline methochloride. These latter observations led to the suggestion that this phase of the action of ibotenic acid might be the result of chemical or enzymatic decarboxylation of ibotenic acid to give muscimol (Fig. 1) within the tissue (Curtis et al., 1979). Lambert et al. (1980) and Engberg et al. (1983) have shown that the biphasic nature of the action of ibotenic acid is a function of the presence of barbiturate anesthetics. 4-Methylibotenic acid [(*RS*)-α-amino-3-hydroxy-4-methyl-5-isoxazoleacetic acid] (Hansen and Krogsgaard-Larsen, 1980) shows an NMA-like excitatory effect less potent than (Krogsgaard-Larsen et al., 1980) but otherwise virtually indistinguishable from that of ibotenic acid, with the notable exception that the excitatory effect of 4-methylibotenic acid is not followed by a depressant phase (Engberg et al., 1983; J. D. C. Lambert, unpublished observation). Because 4-methylmuscimol (4-methyl-5-aminomethyl-3-isoxazolol), the compound formed by decarboxylation of 4-methylibotenic acid, is only a very weak γ-aminobutyric acid (GABA) agonist (Krogsgaard-Larsen et al., 1975), these observations may be consistent with the proposal that the depressant phase of the action of ibotenic acid is caused by its decarboxylated product muscimol (Curtis et al., 1979).

An important factor of this problem evidently is the ability of ibotenic acid to undergo decarboxylation in the absence or presence of appropriate enzymes. Although ibotenic acid is known to be converted into muscimol in the dried mushrooms (Eugster, 1969), these aspects have not been studied in detail. In the present article we describe the results of studies on the chemical and biochemical stability of ibotenic acid and a number of structurally related excitatory amino acids.

MATERIALS AND METHODS

Chemicals

Ibotenic acid was a gift from Dr. C. H. Eugster (Department of Organic Chemistry, University of Zürich, Zürich, Switzerland). The following isoxazole amino acids were synthesized according to published procedures: muscimol (Krogsgaard-Larsen and Christensen, 1976), (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid (7-HPCA) (Krogsgaard-Larsen et al., 1984*a*), 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP) (Krogsgaard-Larsen, 1977), (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (Hansen and Krogsgaard-Larsen, 1980; Honoré and Lauridsen, 1980), 5-methyl-4-(2-aminoethyl)-3-isoxazolol (AEMI) (Hjeds and Krogsgaard-Larsen, 1976), and (*RS*)-α-amino-3-hydroxy-4-bromo-5-isoxazolepropionic acid (4-Br-homoibotenic acid) (Hansen and Krogsgaard-Larsen, 1980). An article on the synthesis of 4-bromo-5-(2-aminoethyl)-3-isoxazolol (4-Br-homomuscimol) (H. Mikkelsen and P. Krogsgaard-Larsen) is in preparation. (*S*)-[U-^{14}C]Glutamic acid (specific activity 260 mCi/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). Pyridoxal-5-phosphate (PALP), 3-mercaptopropionic acid (3MPA), allylglycine, 2-aminoethylisothiouronium bromide hydrobromide (AET), and EDTA were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). All other chemicals used were of analytical grade quality from usual commercial sources. Solvents were distilled before use.

Preparation of homogenates and subcellular fractions

Homogenates of brain and liver were prepared in 50 m*M* potassium phosphate (pH 7.2) containing 1 m*M* AET, 0.1 m*M* EDTA, and 0.2 m*M* PALP. For removal of PALP, the homogenate was dialyzed overnight at 4°C against large volumes of the same buffer without PALP. For preparation of synaptosomes, brains were homogenized in 0.32 *M* sucrose and subsequently centrifuged on Ficoll gradients (Henn, 1980).

Enzyme assays

The activity of (*S*)-glutamic acid decarboxylase (GAD) was determined using the method of Roberts and Simonsen (1963). The buffer consisted of 50 m*M* potassium phosphate (pH 7.2), 1 m*M* AET, 0.1 m*M* EDTA, 0.2 m*M* PALP, 0.1% (vol/vol) Triton X-100, and 3 m*M* (*S*)-[U-^{14}C]glutamic acid, unless otherwise stated. Incubations were carried out at 37°C for 15–30 min. In cases in which ibotenic acid decomposition rates and GAD activities were compared, the same homogenates were used with different substrates (ibotenic and glutamic acid).

The biochemical stability of ibotenic acid was investigated by incubation of ibotenic acid (3 m*M*) with the above-mentioned homogenates of liver and brain at 37°C. Reactions were terminated by addition of perchloric acid, and neutralized samples were analyzed for ibotenic acid and muscimol (see below).

Protein concentrations were determined in aliquots using the conventional method of Lowry et al. (1951) with bovine serum albumin as the standard.

Chemical analysis

A liquid chromatograph consisting of a pump (model 110A; Altex, Berkeley, CA, U.S.A.), a 20-μl loop injector (model 7120 Rheodyne, Berkeley), an absorbance detector (model 750; Kontron, Luton, England) equipped with a 215-nm filter, and an Omniscribe recorder (model 5117-5; Houston Instruments, Austin, TX, U.S.A.) was used. A stainless steel column, 12 cm × 4.6 mm i.d. (Knauer, Berlin, G.F.R.), was packed with LiChrosorb NH_2 (particle size 5 μm; E. Merck, Darmstadt, G.F.R.), using the dilute slurry technique and methanol as the suspension medium.

The content of ibotenic acid (4.6), muscimol (3.3), 7-HPCA (5.4), THIP (3.7), AMPA (2.1), AEMI (1.1), 4-Br-homoibotenic acid (3.0), and 4-Br-homomuscimol (2.1) was estimated by HPLC as exemplified in Fig. 2 (numbers in parentheses are k' values, where $k' = (t_R - t_M)/t_M$). The chromatography was performed at ambient temperature, and separation of the amino acids was achieved by elution with an eluent consisting of acetonitrile, 0.2 *M* potassium phosphate buffer (pH 6.0), and water (60:5:35). The flow rate was 2.0 ml min^{-1} and all the individual separations were performed within 4 min.

All samples were analyzed in duplicates, and the heights of the peaks were measured. Quantitation was performed from individual standard curves for each amino acid.

RESULTS

Chemical stability

Solutions of the excitatory amino acids ibotenic acid, 7-HPCA, AMPA, and 4-Br-homoibotenic acid (Fig. 1) in 0.2 *M* potassium phosphate buffer, pH 2.7, 7.4, and 10.0, were kept at 37°C for 24 h without any observed decomposition.

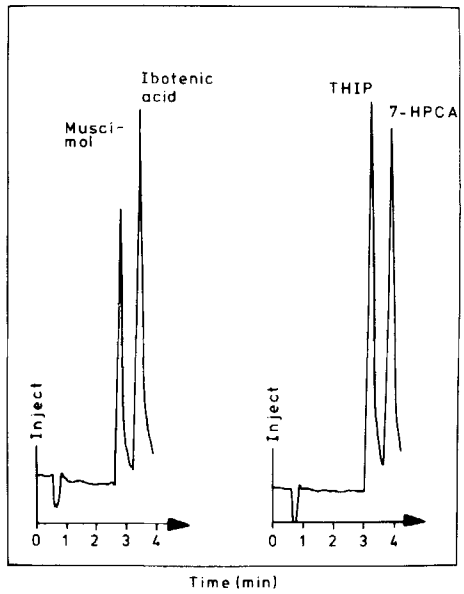

FIG. 2. HPLC separation of muscimol and ibotenic acid and THIP and 7-HPCA, respectively. The column, 12 cm × 4.6 mm, contained LiChrosorb NH_2 (5 μm), the eluent was acetonitrile, 0.2 *M* potassium phosphate buffer (pH 6.0), and water (60:5:35), the flow rate was 2.0 ml/min, and the injection volume was 5 μl (representing 0.5 μg of each compound). The detector was set at a wavelength of 215 nm, and the detector sensitivity was 0.1 absorbance units full scale.

Heating of the solutions of AMPA and 4-Br-homoibotenic acid on a steam bath for 4 h showed that these two compounds were stable over the whole pH range at 100°C as well.

Solutions of ibotenic acid, pH 2.7, 7.4, and 10.0, appeared to be unstable; if stored for 2 h at 100°C, the compound was quantitatively decarboxylated to muscimol at pH 2.7. At pH 7.4 and 10.0, the decarboxylation was both less complete and slower than at pH 2.7, and at pH 10.0 ibotenic acid was only partially transformed into muscimol (Fig. 3).

The effect of pH on the stability of 7-HPCA is shown in Fig. 4. 7-HPCA was quantitatively decarboxylated into THIP in acid solution, and the decarboxylation process was found to be faster than for ibotenic acid. The amount of 7-HPCA was decreased to a small extent at pH 7.4 and 10.0. At pH 10.0, the decomposition of 7-HPCA was only partially followed by production of THIP, and no THIP formation was seen at pH 7.4.

Biochemical stability

All the excitatory amino acids in Fig. 1 were found to be relatively weak inhibitors of the decarboxylation of (*S*)-[U-^{14}C]glutamic acid by GAD in whole mouse brain homogenate, with IC_{50} values of ~1–2 m*M* (Table 1).

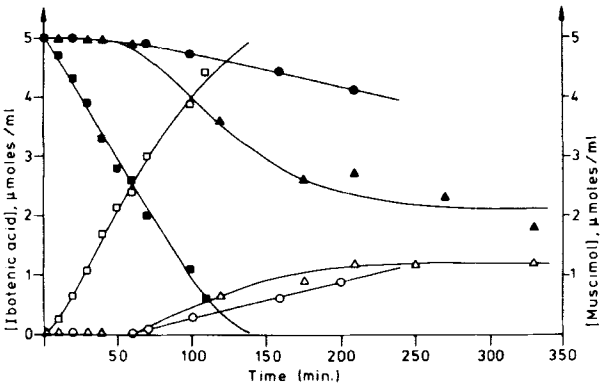

FIG. 3. The chemical stability of ibotenic acid at different pH values and at 100°C. (■) Ibotenic acid decomposition, pH 2.7; (□) muscimol formation, pH 2.7; (●) ibotenic acid decomposition, pH 7.4; (○) muscimol formation, pH 7.4; (▲) ibotenic acid decomposition, pH 10.0; and (△) muscimol formation, pH 10.0. The ordinates are expressed in µmol/ml and the abscissa in min. Each point is the mean of duplicate determinations.

Incubation of the amino acids with a brain homogenate showed that only ibotenic acid could be decarboxylated. None of the other amino acids was transformed into the corresponding decarboxylated products (Fig. 1) or other compounds. Exactly the same result was observed after incubation of the amino acids with a liver homogenate, i.e., only ibotenic acid underwent decarboxylation, with a decomposition rate faster than that observed for the brain homogenate (Table 2).

The rate of decarboxylation of ibotenic acid among subcellular fractions of brain was observed to be highest in the synaptosomal fraction (Table 2). Moreover, the rate of decarboxylation per milligram of protein was ~40 times higher in the synaptosomes than in the whole brain homogenates.

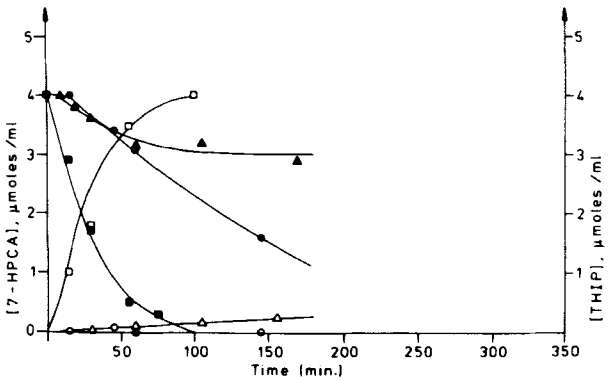

FIG. 4. The chemical stability of 7-HPCA at different pH values and at 100°C. (■) 7-HPCA decomposition, pH 2.7; (□) THIP formation, pH 2.7; (●) 7-HPCA decomposition, pH 7.4; (○) THIP formation, pH 7.4; (▲) 7-HPCA decomposition, pH 10.0; and (△) THIP formation, pH 10.0. The ordinates are expressed in µmol/ml and the abscissa in min. Each point is the mean of duplicate determinations.

TABLE 1. *Inhibition of GAD activity in mouse brain homogenate by some excitatory amino acids*

Inhibitors	IC_{50} (m*M*)
Ibotenic acid	1.5
7-HPCA	1.9
AMPA	2.5
4-Br-homoibotenic acid	1.4

The (*S*)-[U-^{14}C]glutamic acid concentration was 1.0 m*M*, and incubation was for 15 min at 37°C. The concentration of the inhibitors varied in the range 0.3–10 m*M*, and four to five different concentrations (each in triplicate) were used for each inhibitor. IC_{50} values were determined by log-probit analysis of percentage inhibition of GAD activity as a function of the inhibitor concentration (Balcar et al., 1976). SEM values were <15%.

The production of muscimol cannot alone account for the total amount of ibotenic acid being decomposed. In brain, the decomposition rate for ibotenic acid is about five times the formation rate for muscimol, and in liver homogenate, the decomposition rate is about twice the rate of formation of muscimol (data not shown).

In an attempt to elucidate the requirement for PALP of the enzymes responsible for the decomposition processes, homogenates were incubated with ibotenic acid in both the absence and the presence of PALP. The decomposition rate for ibotenic acid was reduced to one-fourth of the control value in the absence of PALP in the brain homogenate. Addition of 0.2 m*M* PALP increased the decomposition rate to almost the control level (Table 3). In the case of the liver homogenate, the decomposition rate was observed to be half the control value in the absence of PALP and almost elevated to the control value by addition of 0.2 m*M* PALP to the incubation mixture (Table 3).

The percentage inhibition of the decomposition of ibotenic acid by aminooxyacetic acid (AOAA), 3MPA, and allylglycine is shown in Table 4. The three compounds were observed to inhibit the decomposition of ibotenic acid in brain homogenate to the same extent by ~40%. In liver homogenate, the decomposition was also inhibited by AOAA and 3MPA, whereas allylglycine had only little effect. (*S*)-Glutamic acid was seen to have some inhibitory

TABLE 2. *Decomposition rates for ibotenic acid by enzymes from different tissue preparations*

Preparation	nmol of ibotenic acid min^{-1} mg^{-1} of protein
Liver	11.8 ± 0.8
Whole brain	8.7 ± 0.5
Mitochondria	252.3 ± 13.5
Synaptosomes	337.3 ± 14.5

The final ibotenic acid concentration in the homogenates was 3.0 m*M*, and incubation was for 30 min at 37°C. Data are mean ± SEM values (n = 3).

effect on the decomposition of ibotenic acid by brain homogenate, but was without inhibitory effect in the liver homogenate assays (Table 5). The inhibitory effect on the AOAA-sensitive decomposition in brain was much more pronounced than the inhibition of the AOAA-insensitive decomposition.

DISCUSSION

Ibotenic acid is a potent excitatory amino acid (Johnston et al., 1968; MacDonald and Nistri, 1978; Curtis et al., 1979; Krogsgaard-Larsen et al., 1980) showing a unique pharmacological profile. The effect of microelectrophoretically administered ibotenic acid on spinal neurons in cats anesthetized with barbiturates is biphasic. It is generally agreed that the initial excitatory effect of ibotenic acid is mediated primarily by NMA receptors (Curtis et al., 1979; Lambert et al., 1980; Watkins and Evans, 1981; Engberg et al., 1983; McLennan, 1983; Foster and Fagg, 1984), but the mechanisms underlying the prolonged, but reversible, inhibitory phase, during which the sensitivity of the neurons to excitation by ibotenic acid or other excitants is reduced, have not been clarified yet. This biphasic effect may be an inherent property of ibotenic acid (MacDonald and Nistri, 1978; Nistri and Constanti, 1979), or, alternatively, a consequence of the formation of the potent GABA agonist muscimol by decarboxylation of ibotenic acid within the tissue (Curtis et al., 1979; Engberg et al., 1983). In an attempt to shed some light on these problems, we have studied the ability of ibotenic acid to undergo decarboxylation under different conditions in the absence or presence of appropriate enzymes.

The stability of ibotenic acid at 37°C in the absence of enzymes was studied using HPLC analytical techniques. Under these conditions and at different pH values (2.7, 7.4, or 10.0), ibotenic acid did not undergo detectable decomposition during a 24-h period. At 100°C and pH 7.4, ibotenic acid was slowly decomposed with an estimated half-life of 10 h and with concomitant formation of muscimol (Fig. 3), a process which was accelerated at pH 10.0 and, in particular, at pH 2.7 (estimated half-life 60 min; Fig. 3). These results emphasize that test solutions of ibotenic acid should be prepared with great care, but it is evident that under physiological conditions, negligible amounts of ibotenic acid will undergo spontaneous decarboxylation to give muscimol.

7-HPCA, which is a potent and highly selective agonist at quisqualic acid-preferring receptors (Krogsgaard-Larsen et al., 1984*a*), is an analogue of ibotenic acid of even further reduced conformational mobility (Krogsgaard-Larsen et al., 1984*b*). In accordance with the structural similarity between these compounds (Fig. 1), their apparent rates of decarboxylation are similar. At 37°C and at different pH values, 7-HPCA was not significantly decomposed, whereas at 100°C 7-HPCA underwent decarboxylation to give primarily THIP at rates (Fig. 4) slightly higher than those observed for ibotenic acid. Neither AMPA nor 4-Br-homoibotenic acid, which like 7-HPCA are highly selective agonists at quisqualic acid-preferring receptors (Krogsgaard-Larsen et al., 1980; McLennan, 1983; Foster and Fagg, 1984), was subjected to any detectable decarboxylation under the conditions studied.

TABLE 3. *Effects of PALP on the decomposition rate for ibotenic acid*

Preparation	nmol of ibotenic acid min^{-1} mg^{-1} of protein		
	Control	−PALP	+PALP
Liver	11.8 ± 0.8	5.8 ± 0.3	9.3 ± 0.1
Whole brain	8.7 ± 0.5	1.8 ± 0.4	6.6 ± 0.3

The final concentrations in the homogenates of ibotenic acid and PALP were 3.0 and 0.2 m*M*, respectively. Incubation time was 30 min at 37°C. Data are mean ± SEM values (n = 3).

TABLE 4. *Effects of some inhibitors on the activity of GAD in brain and the decomposition of ibotenic acid in brain and liver*

Inhibitors	Inhibition (%)		
	Ibotenic acid decomposition		GAD brain
	Liver	Brain	
AOAA (0.1 m*M*)	100	41	100
3MPA (0.1 m*M*)	100	42	100
Allylglycine (1.0 m*M*)	30	43	67

The activity of GAD and the decomposition of ibotenic acid were determined at substrate concentrations of 3.0 m*M*. For decomposition of ibotenic acid in liver and brain, the incubation time was 30 min, and for GAD assays, the incubation time was 15 min. All incubations were carried out at 37°C. Results are averages of three to five individual experiments, and SEM values were <15%.

TABLE 5. *Inhibition of the decomposition of ibotenic acid in liver and brain by (S)-glutamic acid*

Decomposition condition	IC_{50} (m*M*)
Liver	No inhibition
Brain (AOAA-sensitive + -insensitive)	60
Brain (sensitive to 0.1 m*M* AOAA)	3.5

The concentration of ibotenic acid was 3.0 m*M*, and incubation was for 30 min at 37°C. The concentration of (*S*)-glutamic acid varied in the range 1–30 m*M*, and five different concentrations (each in triplicate) were used to determine IC_{50} values from log-probit analysis of percentage inhibition as a function of the (*S*)-glutamic acid concentration (Balcar et al., 1976). SEM values were <15%.

Although all the excitatory amino acids listed in Fig. 1 were relatively weak inhibitors of the activity of GAD (Table 1), only ibotenic acid underwent decomposition in the presence of mouse brain homogenates. In an attempt to shed some light on the mechanism(s) underlying the decomposition of ibotenic acid in the presence of brain homogenate, the rate of which (8.7 nmol min^{-1} mg^{-1} of protein) was higher than that measured for (*S*)-glutamic acid (3.2 nmol min^{-1} mg^{-1} of protein), the effects of various inhibitors were studied. Removal of PALP from the incubation mixture reduced the rate of decomposition of ibotenic acid by ~80% (Table 3), and, furthermore, the decomposition process was sensitive to 3MPA, allylglycine, AOAA, and (*S*)-glutamic acid (Tables 4 and 5).

In light of the absolute requirement of GAD for PALP and the sensitivity of the activity of this enzyme to substrate inhibition and inhibition by 3MPA, allylglycine, and AOAA (Alberici et al., 1969; Wu and Roberts, 1974), these studies seem to indicate that GAD plays a role in the decomposition of ibotenic acid under the in vitro conditions described. Such a role of GAD is further substantiated by the observation that the synaptosomal fraction, which contains the GAD activity (Weinstein et al., 1963), was enriched in ibotenic acid decomposition activity. It must, however, be emphasized that the decomposition of ibotenic acid is only partially accompanied by the formation of muscimol. This observation may reflect that decarboxylation is not the only reaction responsible for the decomposition of ibotenic acid in mouse brain homogenates, in agreement with the finding that the rate of decomposition of ibotenic acid is twice as high as that of (*S*)-glutamic acid. It should, however, be kept in mind that during the incubation some of the muscimol formed could undergo a transamination process (Fowler et al., 1983).

Similarly, decarboxylation to give muscimol apparently is only one of the mechanisms for the decomposition of ibotenic acid in mouse liver homogenates. Under these conditions, the decomposition of ibotenic acid was also inhibited by 3MPA and AOAA, whereas allylglycine had little effect (Table 4). However, dialysis of the liver homogenate to remove PALP only partially inhibited the decomposition of ibotenic acid, suggesting that the presence of PALP is not an absolute requirement for the enzyme(s) responsible for the decomposition of ibotenic acid under these conditions. GAD is known not to be present in the liver (Wu et al., 1978), and on the basis of the present studies, it is not possible to draw conclusions with respect to the mechanism(s) underlying the decomposition of ibotenic acid in the liver. One possibility might be that in the liver, which possesses extremely active aminotransferases, ibotenic acid could undergo transamination before decarboxylation. Such a mechanism would be compatible with the finding that AOAA and 3MPA, which interfere with some aminotransferases (Schousboe et al., 1974), inhibited the decomposition of ibotenic acid in liver homogenates.

In summary, the present studies have shown that under the conditions studied and in the absence of enzymes, ibotenic acid does not undergo significant decomposition at physiologically relevant temperatures and pH values. On the other hand, in brain homogenates, and probably also in intact or less disintegrated brain tissues, ibotenic acid is unstable and muscimol is the major decomposition product. These aspects must be taken into consideration in the analyses of the excitatory/inhibitory or neurotoxic effects of ibotenic acid on central neurons.

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