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L-BOAA induces selective inhibition of brain mitochondrial enzyme, NADH-dehydrogenase

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Lathyrism, a human neurological disorder has been linked to the excessive consumption of a plant toxin, β-oxalylamino-L-alanine (L-BOAA) present in *Lathyrus sativus*. The present study was carried out to elucidate the biochemical mechanisms underlying L-BOAA-induced toxic insult. Incubation of sagittal slices of mouse brain with L-BOAA resulted in dose and time-dependent inhibition of mitochondrial NADH-dehydrogenase (NADH-DH). Significant inhibition of NADH-DH was seen following incubation of brain slices with very low concentration of L-BOAA (0.1 pM). L-BOAA also induced lactate dehydrogenase (LDH) leakage from the slice into the medium in dose-dependent manner. The inhibition of NADH-DH preceded LDH leakage from the slices into the medium. L-BOAA had no effect on other mitochondrial enzymes, namely, isocitrate dehydrogenase or cytochrome *c* oxidase. Incubation of isolated mouse brain mitochondria with L-BOAA also resulted in inhibition of NADH-DH. L-BOAA-induced inhibition of NADH-DH was prevented by non-*N*-methyl-D-aspartate (non-NMDA) glutamate receptor antagonists in general and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor antagonist (NBQX) in particular. Other glutamate agonists examined namely, *N*-methyl-D-aspartate, β-*N*-methylamino-L-alanine (L-BMAA), L-glutamic acid, *N*-acetylaspartylglutamate (NAAG), quisqualic acid, kainic acid or AMPA did not have any effect on NADH-DH activity in slices although they induced LDH leakage from the slice into the medium. Incubation of brain slices with L-BOAA did not induce lipid peroxidation or changes in glutathione levels. Prior incubation of slices with glutathione (GSH) or GSH-isopropyl ester did not prevent L-BOAA-induced inhibition of NADH-DH. However, incubation of isolated mitochondria with L-BOAA in the presence of GSH-isopropyl ester prevented L-BOAA-induced inhibition of NADH-DH, indicating the protective effect of mitochondrial glutathione in the prevention of L-BOAA-induced toxicity.

INTRODUCTION

The study of molecular mechanisms underlying neurodegenerative disorders has been an area of extensive investigation. Ingestion of neurotoxic excitatory amino acid, L-BOAA[36], present in *Lathyrus sativus*[24], has been implicated in human neurodegenerative disorder 'lathyrism'[13,32]. Administration of L-BOAA to primates elicited motor neuron disorder similar to that seen in humans[32]. Brain and retinal degeneration have been reported following administration of L-BOAA to mouse[19]. Postsynaptic vacuolation and dark, shrunken cells were seen after incubation of explant cultures of mouse brain and spinal cord with L-BOAA[17,27]. Considerable efforts have been made to understand the mechanism of the toxic action of L-BOAA. Non-NMDA class of glutamate receptor antagonists selectively block L-BOAA induced toxic insult both in vitro and in vivo indicating that L-BOAA probably acts through excitatory amino acid receptors[26,27,38]. However, the biochemical sequelae leading to toxic damage following receptor activation is still unclear.

The present study was carried out to evaluate molecular mechanisms underlying L-BOAA induced toxicity in mouse brain slices and to compare it with other glutamate receptor agonists.

Sagittal slices of rodent brain provide a good in vitro model for mechanistic evaluation of neurotoxicity. The selectivity and sensitivity of this model has been established using a variety of neurotoxins[20,21,25] including excitatory amino acid[20]. Hence, this in vitro model was chosen for the present study.

The generation of oxidative stress by excitotoxic amino acids has been demonstrated[12]. The generation

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of oxidative stress would result in loss of protein thiols leading to inhibition of vital enzymes which contain the thiol groups in their active site. More recently, the vital mitochondrial enzyme, NADH-dehydrogenase has been shown to be inhibited following exposure to reactive oxygen species[41]. The present study proposes to examine the effect of L-BOAA on the above enzyme using an in vitro model consisting of sagittal slices of mouse brain.

MATERIALS AND METHODS

Materials

L-BOAA, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 6,7-dinitroquinoxaline-2,3-dione (DNQX), 5-methyl-10,11-dehydro-5H-benzo (α, δ) cyclohepten-5-10-imine maleate [(+)MK-801)], NMDA, AMPA, kainic acid and L-BMAA were obtained from Research Biochemical Inc., USA. Glutamate diethyl ester (GDEE) was procured from Sigma Chemical Co., USA. 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) was a gift from Novo Nordisk, Denmark. Glutathione (GSH)-isopropyl ester was a kind gift from Yamanouchi Pharmaceuticals Co. Ltd., Japan.

Methods

Swiss Albino mice (3–4 months old) weighing 25–30 g were used for all experiments. Animals were obtained from NIMHANS Central Animal Research Facility and had free access to pelleted diet (Lipton India Ltd., Bombay) and water ad libitum. Following decapitation, brains were quickly removed and sagittal slices of uniform thickness (0.5 mm) were prepared using a slicer[22]. Four sagittal slices were obtained from each mouse brain. Brain slices were incubated in artificial cerebrospinal fluid (ACSF, pH 7.4; 1.5 ml/slice) containing (in mM): NaCl 124, KCl 5, $MgSO_4$ 1.3, $CaCl_2$ 1.3 glucose 10, KH_2PO_4 1.2 and glycylglycine 30 or $NaHCO_3$ 25, with and without L-BOAA. Incubations were carried out in an atmosphere of oxygen at 37°C. In some experiments, incubations were carried out in ACSF in which magnesium was absent, but was otherwise similar. To study the effect of glutathione (GSH) or GSH-isopropyl ester on L-BOAA toxicity, slices were preincubated with GSH (10 mM) or GSH-isopropyl ester (1 mM) for 30 min prior to addition of L-BOAA (10 pM). At the end of the incubation period, slices were removed, rinsed and homogenized in 0.32 M sucrose. The homogenate was centrifuged at 1,000 × *g* for 10 min to remove cell debris. The supernatant was used for the assay of enzyme activity. Following incubation, the ACSF was filtered and used for the estimation of LDH activity.

Measurement of NADH-DH activity. Brain homogenate was freeze thawed twice and 90–120 μg of the protein was used for the assay. The assay mixture consisted of phosphate buffer (0.05 M, pH 7.6) containing sucrose (0.25 M) and the brain homogenate. The reaction was initiated by the addition of NADH (0.28 mM) and the rate of change of absorbance at 340 nm was monitored as described[21,23].

Assay of LDH. The leakage of LDH from the slices into the medium following incubation with excitatory amino acids was measured as described[20,21]. Twenty microliters of ACSF medium was added to 980 μl of phosphate buffer (0.05 M, pH 7.4) containing 0.65 mM pyruvate and 11.33 mM NADH and the change in absorbance was monitored at 340 nm.

Activities of isocitrate dehydrogenase (ICDH), cytochrome *c* oxidase were estimated as described[25].

Glutathione was measured by enzymatic recycling method[1] and malondialdehyde was measured as thiobarbituric acid reactive products[18]. Protein was estimated using a dye-binding method[2].

The brain slices were preincubated with glutamate receptor antagonists, namely, (+)MK-801 (1 nM), GDEE (100 μM), DNQX (1 μM), CNQX (1 μM) or NBQX (1 μM) for 30 min at 37°C. Following preincubation, L-BOAA (1 pM) was added and the incubation was continued for 1 hr. The effect of NBQX or CNQX on L-BOAA-induced damage was also examined by injecting NBQX or CNQX (30 mg/kg body weight), subcutaneously to mice. The animals were sacrificed 4 h after the injection of quinoxalinediones and sagittal slices of brain were prepared from the quinoxalinedione treated mice. The slices were incubated with and without L-BOAA (10 pM) for 1 h at 37°C. Similar experiments were also carried out using brain slices from untreated mice.

Isolation of mitochondria. Mouse brain mitochondria was isolated as described by Sims[31] with certain modifications. Animals were anaesthetized prior to decapitation with ether and perfused transcardially with ice cold buffer (0.1 M Tris-HCl containing 1.15% KCl, pH 7.4). The brains were rapidly removed and the mitochondrial fraction was isolated using Percoll gradient. Isolation medium contained 0.32 M sucrose, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 0.1 mM 1,4-dithio-D,L-threitol in 10 mM Tris-HCl (pH 7.4). The mitochondrial pellet obtained using the above procedure was relayered on a similar discontinuous Percoll density gradient and the process was repeated to obtain pure mitochondria. The mitochondria was suspended in incubation medium consisting of potassium phosphate buffer (3 mM, pH 7.4) containing HEPES (4 mM), EDTA (1 mM), sucrose (0.25 M), malic acid (100 μM), glutamic acid (100 μM) and mercaptoethanol (5 mM). Freshly isolated mitochondria were incubated with L-BOAA (10 pM), GSH-isopropyl ester (1 mM) or GSH-isopropyl ester (1 mM) and L-BOAA (10 pM) for one hour at 37°C in

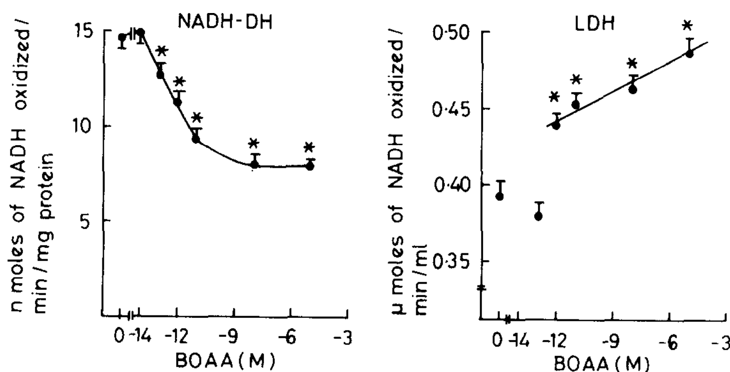

Fig. 1. Dose-dependent effect of L-BOAA on NADH-DH activity in slices and the leakage of LDH from mouse brain slices into the medium. Mouse brain slices were incubated with various concentrations of L-BOAA for 1 h at 37°C under an atmosphere of oxygen. Following incubation, NADH-DH activity was measured in slice homogenate and leakage of LDH from the slices into the medium was monitored. Values are mean ± S.D. (n = 4–8). Asterisks represent values significantly different from controls ($P < 0.05$).

an atmosphere of oxygen. After the incubation, NADH-DH activity was measured in the mitochondrial suspension.

Statistical analysis was carried out using students *t*-test or Analysis-of-Variance (ANOVA) with Duncan's test where appropriate.

RESULTS

Incubation of mouse brain slices with various concentrations of L-BOAA resulted in dose-dependent decrease of NADH-DH activity (Fig. 1). Significant inhibition was noted when the slices were incubated with 0.1 pM L-BOAA. NADH-DH activity decreased thereafter in a dose-dependent manner and maximum inhibition (52%) was observed when L-BOAA concentration in the incubation medium was 10 μM. Incubation of mouse brain slices with L-BOAA also resulted in dose-dependent leakage of LDH from the slice into the medium (Fig. 1). Significant leakage of LDH from the slice into the medium was seen when L-BOAA concentration in the medium was 1 pM indicating that NADH-DH inhibition preceded LDH leakage.

Mouse brain slices were incubated for various time intervals (15–60 min) with and without 10 pM L-BOAA (Fig. 2). L-BOAA-induced inhibition of NADH-DH activity (16%) in slices was observed following 30 min incubation (Fig. 2). When the slices were incubated with L-BOAA for longer periods (60 min), enhanced inhibition of NADH-DH (36%) was observed.

In order to determine if L-BOAA had any effect on other mitochondrial enzymes, higher concentrations of L-BOAA (10 pM–10 μM) were added to the ASCF medium containing mouse brain slices and incubated for 1 h at 37°C under oxygen. Even after incubation of slices with a very high concentration of L-BOAA (10 μM), the activity of other mitochondrial enzymes,

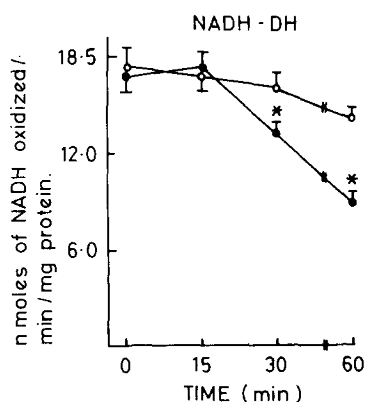

Fig. 2. Effect of incubation time on L-BOAA induced NADH-DH inhibition in brain slices. Mouse brain slices were incubated with L-BOAA (10 pM) for 15, 30 and 60 min. NADH-DH activity in the slice was monitored following the incubation. Slices incubated without L-BOAA served as controls. Values are mean ± S.D. ($n = 4$–6). Asterisks represents values significantly different from corresponding controls ($P < 0.05$).

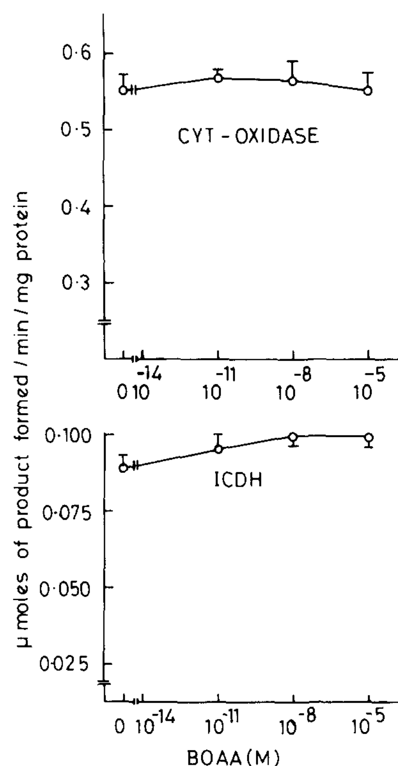

Fig. 3. Effect of L-BOAA on isocitrate dehydrogenase and cytochrome *c* oxidase activity. Isocitrate dehydrogenase and cytochrome *c* oxidase activity were measured in the slice homogenate following incubation of mouse brain slices with various concentrations of L-BOAA for 1 h at 37°C. Values are mean ± S.D. ($n = 4$).

namely, isocitrate dehydrogenase (ICDH) and cytochrome *c* oxidase remained unaffected as shown in Fig. 3. However, the activities of the lysosomal enzymes, *N*-acetylglucosaminidase and acid phosphatase were inhibited significantly after incubation with 10 pM L-BOAA (data not shown).

Mouse brain slices were preincubated with glutamate receptor antagonists, namely, (+)MK-801 (NMDA receptor antagonist) or GDEE (non-NMDA receptor antagonist) for 30 min at 37°C. Following preincubation, L-BOAA (1pM) was added to one set of slices and the incubation was continued further for 1 h. GDEE completely protected the slices against the toxic action of L-BOAA by preventing inhibition of NADH-DH, while (+)MK-801 had no effect even at high concentration (100 nM; Table I). In order to test the efficacy of other non-NMDA receptor antagonists (namely quinoxalinediones) in protecting against L-BOAA induced toxicity, NBQX or CNQX were administered subcutaneously to mice (30 mg/kg body weight) and one set of slices prepared from drug treated animals were incubated with L-BOAA (10 pM) for 1 h at 37°C. The other set served as control. Both NBQX and CNQX protected against L-BOAA induced inhibition of NADH-DH (Table I). CNQX and NBQX did not

protect the slices from L-BOAA-induced toxicity when added in vitro. However, preincubation of mouse brain slices with DNQX (1 μM) for 30 min at 37°C protected the slices against L-BOAA-induced inhibition of NADH-DH (Table I).

To examine the effect of other glutamate receptor agonists on NADH-DH activity, mouse brain slices were incubated with NMDA, L-BMAA, L-glutamic acid, NAAG, quisqualic acid, kainic acid or AMPA for 1 h at 37°C. None of the glutamate agonists examined inhibited NADH-DH activity (Table II). The minimum toxic dose of glutamate agonists required to induce significant LDH leakage is also given in Table II. The doses of agonists used were substantially higher than the minimum toxic dose in all cases, except L-BMAA.

The effect of varying concentration of L-BOAA on the levels of glutathione and lipid peroxidation products (measured as malondialdehyde) in brain slices is depicted in Table III. Incubation with L-BOAA did not result in any significant change in the levels of glutathione or malondialdehyde in the slices.

The effect of prior incubation of brain slices with GSH or GSH-isopropyl ester on L-BOAA-induced inhibition of NADH-DH is described in Table IV. Both GSH and GSH-isopropyl ester failed to protect the slices from L-BOAA-induced inhibition of NADH-DH. Prior incubation with GSH or GSH-isopropyl ester alone had no effect on the NADH-DH activity in brain slices.

TABLE I

Effect of glutamate receptor antagonists on L-BOAA induced inhibition of NADH-DH activity in mouse brain slices

	NADH-DH activity	
	− BOAA	*+ BOAA*
Control	14.5±0.2	12.1±0.4 *
NMDA antagonist		
MK-801 (1 nM)	13.4±0.7	10.6±0.1 *
MK-801 (100 nM)	13.6±0.1	10.4±1.8 *
Non-NMDA antagonists		
GDEE (100 μM)	14.5±0.7	14.0±1.0
DNQX (1 μM)	14.5±0.8	13.7±1.2
CNQX (1 μM)	14.2±1.6	11.2±1.7 *
CNQX (30 mg/kg body wt. s.c.)	14.5±0.3	14.2±0.9
NBQX (1 μM)	14.4±0.6	12.4±0.5 *
NBQX (30 mg/kg body wt. s.c.)	14.7±1.2	15.0±0.1

Mouse brain slices were preincubated with MK-801, GDEE, CNQX, DNQX or NBQX for 30 min at 37°C. The concentration of the antagonists used is given in parenthesis. Following preincubation, L-BOAA (1 pM) was added to one set of slices and the incubations were continued further for a period of 1 h. CNQX or NBQX were injected (30 mg/kg body weight) to mice subcutaneously (s.c) and sacrificed 4 h later. Brain slices prepared from treated mice were incubated with L-BOAA (10 pM). Slices from untreated animals were also incubated simultaneously. NADH-DH activity was measured in the slice homogenate at the end of incubation period and is expressed as nmoles of NADH oxidized/min/mg protein. Values are mean ± SD (n = 4–6). Asterisks represent values significantly different from corresponding controls ($P < 0.05$).

TABLE II

Effect of various glutamate receptor agonists on NADH-DH activity in mouse brain slices

Agonist	*NADH-DH activity*	*Minimum dose needed for significant leakage of LDH*
None (Control)	14.7±1.2	–
L-BOAA (0.1 pM)	12.8±0.7 *	1.0 pM
L-Glutamate (1 mM)	14.2±1.2	10 μM
NAAG (1 μM)	14.9±1.4	0.1 pM
L-BMAA (1 mM)	14.0±1.2	1 mM
NMDA (1 μM)	15.0±1.1	1 nM
Quisqualic acid (1 μM)	14.4±0.9	1 nM
AMPA (1 mM)	15.5±1.1	100 μM
Kainic acid (1 mM)	14.4±0.9	1 μM

Mouse brain slices were incubated with L-BOAA, L-glutamate, NAAG, L-BMAA, NMDA, quisqualic acid, AMPA and kainic acid for 1 h at 37°C. NADH-DH activity was measured in the slice homogenate following incubation and is expressed as nmol of NADH oxidized/min/mg protein. Values are mean ± SD (n = 4–8). Asterisks represent values significantly different from controls ($P < 0.05$).

TABLE III

The effect of L-BOAA on the concentration of glutathione and malondialdehyde in brain slices

	GSH (μmol / g tissue)	*Malondialdehyde (nmol / g tissue)*
Control	1.74±0.2	46.3±3.1
L-BOAA (1 pM)	1.57±0.1	43.7±4.9
L-BOAA (1 nM)	1.73±0.2	50.3±2.6
L-BOAA (1 μM)	1.69±0.2	51.0±5.4

Brain slices were incubated with various concentrations of L-BOAA (1 pM–1 μM) for 1 h at 37°C. Following incubation the concentrations of GSH and malondialdehyde were measured in slices. Values are mean ± S.D. (n = 4–6).

TABLE IV

Effect of preincubation of brain slices with glutathione or glutathione-isopropyl ester on L-BOAA induced NADH-DH inhibition

	NADH-DH (% control)
Control	100 ±6.1
L-BOAA	80.7±5.4 *
GSH	99.4±3.3
GSH + L-BOAA	80.0±4.9 *
GSH – isopropyl ester	101.4±4.7
GSH – isopropyl ester + L-BOAA	81.9±6.4 *

Mouse brain slices were preincubated with GSH (10 mM) or GSH-isopropyl ester (1 mM) for 30 min at 37°C. L-BOAA (10 pM) was added to one set of slices and the incubation was further continued for 1 h. Slices incubated in ACSF alone were also run simultaneously. The NADH-DH activity in slices incubated in ACSF was 15.7 ± 0.7 nmol/min/mg protein. Values are mean ± S.D. (n = 4–8). Asterisks represent values significantly different from corresponding controls ($P < 0.05$).

TABLE V

Effect of L-BOAA on NADH-DH activity in isolated mouse brain mitochondria

	NADH-DH activity
Control	38.6 ± 3.2
L-BOAA	29.6 ± 1.8 *
GSH-isopropyl ester	38.6 ± 0.8
GSH-isopropyl ester + L-BOAA	37.9 ± 0.9

Freshly isolated mitochondria from mouse brain were incubated with L-BOAA (10 pM), GSH-isopropyl ester (1 mM) or GSH-isopropyl ester (1 mM) and L-BOAA (10 pM) for 1 h at 37°C in an atmosphere of oxygen. Following incubation, mitochondria were freeze thawed twice and used for NADH-DH assay. NADH-DH activity is expressed as nmoles of NADH oxidized/min/mg protein. Asterisks represent values significantly different from controls ($P < 0.05$). Values are mean ± S.D. ($n = 6$).

Incubation of freshly isolated mitochondria with L-BOAA (10 pM) for 1 h at 37°C resulted in significant inhibition (23%) of NADH-DH activity (Table V). Addition of GSH-isopropyl ester to the incubation medium abolished L-BOAA-induced inhibition of NADH-DH activity in the mitochondria (Table V).

DISCUSSION

The present study demonstrates the selective inhibition of mitochondrial enzyme, NADH-DH by L-BOAA, in vitro, in mouse brain slices. Incubation of mouse brain slices with L-BOAA resulted in dose and time-dependent inhibition of NADH-DH activity (Figs. 1 and 2). NADH-DH activity was inhibited in the slices following incubation with very low concentrations of L-BOAA (0.1 pM). The study of dose-dependent effects indicated that the inhibition of NADH-DH preceded the leakage of cytosolic enzyme LDH, suggesting that mitochondrial damage occurred prior to LDH leakage. The inhibition of NADH-DH by L-BOAA was selective, as other mitochondrial enzyme activities, namely isocitrate dehydrogenase and cytochrome *c* oxidase remained unaffected following incubation of mouse brain slices with very high concentrations of L-BOAA (10 μM, Fig. 3). Inhibition of NADH-DH activity was also observed following incubation of freshly isolated mouse brain mitochondria with L-BOAA (Table V). The maximum inhibition of NADH-DH observed was 51% after exposure to 10 μM L-BOAA. Further increase in the concentrations of L-BOAA did not result in greater inhibition of NADH-DH. These unusual kinetics are observed with the most neurotoxins[20,21] which exert their action on selective populations of cells within specific regions of the brain.

Mouse brain slices were incubated with various glutamate receptor agonists, namely, NMDA[16], L-BMAA[33], L-glutamate[35], NAAG[5,39], quisqualic acid[9], kainic acid[29] or AMPA[7,10] to determine their effect on NADH-DH activity (Table II). NMDA was found to have no effect on NADH-DH activity following incubation with brain slices. Incubations with NMDA were carried out in ACSF with and without magnesium and incubations with L-BMAA were carried out in ACSF which contained sodium bicarbonate or glycylglycine. L-BMAA, (a structural analogue of L-BOAA), has been implicated in the pathogenesis of Guam amyotrophic lateral sclerosis-Parkinson's dementia complex[33]. L-BMAA had no effect on NADH-DH activity. Similar results were also observed with L-glutamate or NAAG, an endogenous excitatory dipeptide, recently shown to possess neurotoxic effects[20]. Kainic acid, quisqualic acid, and AMPA (known to activate kainate and AMPA receptors, respectively) also did not inhibit NADH-DH activity. These results demonstrate that L-BOAA selectively inhibits NADH-DH activity in mouse brain slices and that the mechanism by which L-BOAA exerts its toxic action may be different from that of other glutamate agonists.

Excitatory amino acids mediate their toxic action by acting on the NMDA or non-NMDA class of glutamate receptors[4,15]. Excitotoxicity of L-BOAA is mediated via the non-NMDA class of glutamate receptors[27,38]. Inhibition of L-BOAA toxicity by various non-NMDA receptor antagonists and receptor binding studies have been used to demonstrate that the mechanism of action of L-BOAA probably involves activation of AMPA receptors[27,28,38]. In the present study, GDEE, a non specific non-NMDA receptor antagonist[37], protected L-BOAA-induced inhibition of NADH-DH activity. NMDA receptor antagonist (+)MK-801[34] did not have any effect on L-BOAA-induced mitochondrial toxicity even at high concentration (100 nM). Recently quinoxalinediones, a new class of drugs known to selectively block quisqualate/AMPA receptors have been used to differentiate NMDA and non-NMDA receptor mediated responses[8,30]. Quinoxalinediones, namely, CNQX, DNQX and a more selective AMPA antagonist NBQX, were used in the present study to observe their effect on L-BOAA-induced inhibition of NADH-DH activity. NBQX and CNQX protected the slices against L-BOAA-induced NADH-DH inhibition, when administered in vivo (Table I). However, these drugs did not offer protection to the slices against L-BOAA-induced toxicity when added in vitro. But, DNQX protected the slices against L-BOAA-induced toxicity when added in vitro. The reason for the differential effect of the quinoxalinediones is unclear. Earlier studies on the effect of NBQX in protecting against ischemic injury also involved injection of NBQX, in vivo[30]. Neverthe-

less, experiments with quinoxalinediones clearly demonstrate that L-BOAA-induced inhibition of NADH-DH could be abolished by the quinoxalinediones. The glutamate antagonists used in the present study namely, GDEE, CNQX, DNQX and NBQX did not have any effect on the NADH-DH activity in the slices when incubated alone with the brain slices.

GSH, a major cellular protectant, maintains the thiol homeostasis in the cells[14]. Alteration in GSH levels may lead to membrane damage caused by free radicals[6]. In addition, depletion of GSH levels may also lead to oxidation of free thiol groups in the active sites of enzymes to disulfide linkages thereby inactivating the enzyme[40]. Involvement of oxidative stress in glutamate toxicity has been documented[12]. Since, NADH-DH contains cysteine residues in its active site[3], oxidation of these thiol groups could result in inhibition of enzyme activity as has been recently demonstrated in isolated mitochondria[41]. Hence, in order to determine if such peroxidative processes (where thiols are oxidized to disulphides) were involved in the inhibition of NADH-DH by L-BOAA, we examined the status of GSH following exposure of slices to L-BOAA. Incubation of mouse brain slices with very high concentrations of L-BOAA (1 μM) did not alter GSH levels in slices. There was no increase in malondialdehyde levels (indicative of lipid peroxidation) in L-BOAA (1 μM) treated slices (Table III). Further, prior incubation with GSH or GSH-isopropyl ester failed to protect the brain slices from L-BOAA-induced toxicity. However, addition of GSH-isopropyl ester to isolated mouse brain mitochondrial suspension prevented L-BOAA-induced inhibition of NADH-DH (Table V). Thus, addition of GSH or GSH-isopropyl ester to slices may not have an effect on the mitochondrial GSH levels. The observations made with isolated mitochondria demonstrate that GSH-isopropyl ester can abolish the mitochondrial toxicity of L-BOAA.

Rat (Wistar, 3 months old) brain slices were incubated with various concentrations of L-BOAA. Significant increase in the leakage of LDH from the slices was observed only when the concentration of the toxin in the incubation medium was 1 nM, that is a thousand-fold higher than the least toxic dose required to produce similar response in mouse brain slices. There was no inhibition of NADH-DH activity even at high concentration of L-BOAA (1 μM, data not shown). These results demonstrate that there may be difference in the vulnerability of various species to the toxic action of L-BOAA. Similar effects between mice and rat have also been observed with MPTP, wherein the toxicity of MPTP, mediated via its metabolite MPP^+ by inhibition of NADH-DH, is observed in mice but not in rats[11]. Further, the time course of inhibition of NADH-DH by both MPTP[21] and by L-BOAA are similar, wherein significant inhibition of the enzyme is observed only after 30 min of incubation.

The present study demonstrates that L-BOAA selectively inhibits mitochondrial NADH-DH, a property not shared by other glutamate agonists that were tested. This toxic effect was selectively blocked by non-NMDA receptor antagonists in general and AMPA receptor antagonist in particular. It may be presumed that L-BOAA acts through a novel non-NMDA class of glutamate receptor, which is sensitive to quinoxalinediones. However, this speculation is yet to be substantiated. Nevertheless, the excitotoxic mechanism underlying L-BOAA toxicity are probably not similar to other glutamate agonists that act through both NMDA and non-NMDA class of glutamate receptors.

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