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L-β-Methylamino-Alanine-Induced Behavioral Changes in Rats

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MATSUOKA, Y., Z. RAKONCZAY, E. GIACOBINI AND D. NARITOKU. L-β-Methylamino-alanine-induced behavioral changes in rats. PHARMACOL BIOCHEM BEHAV 44(3) 727–734, 1993.—L-β-*N*-methylamino-L-alanine (L-BMAA, 500 μg) infusions into the lateral ventricle induced splay, clonic convulsions, and rigidity in about 60% of rats. Electroencephalograph (EEG) recording during clonic convulsions and rigidity demonstrated epileptiform discharges. Duration and severity of L-BMAA-induced clonic convulsions were reduced significantly by DNQX, a non-NMDA glutamate receptor antagonist, but not by AP-5, a NMDA receptor antagonist or MK-801, a noncompetitive NMDA antagonist. Latency of L-BMAA-induced clonic convulsions was significantly prolonged by DNQX, AP-5 and MK-801. L-BMAA-induced splay was not modified by DNQX or AP-5 but was slightly enhanced by MK-801. L-BMAA-induced rigidity was abolished by MK-801 and partially inhibited by DNQX and AP-5. The L-BMAA-induced behaviors of grooming, facial tremor, etc. were affected by DNQX, AP-5, and MK-801. Our results suggest that L-BMAA may induce behavioral changes by acting upon several subtypes of excitatory amino acid receptors.

Clonic convulsion L-β-Methylamino-alanine Excitatory amino acid receptor NMDA-type glutamate receptor
Non-NMDA-type glutamate receptor Quisqualate/AMPA-type glutamate receptor Kainate-type glutamate receptor
2-Amino-5-phosphonopentanoic acid MK-801 6,7-Dinitroquinoxaline-2,3-dione

THE naturally occurring amino acid L-β-methylamino-alanine (L-BMAA) is a neurotoxin found in the seed of the false sago palm *Cycas circinalis,* which has been used in foods and medicines by Chamorro residents of Guam or Rota. This uncommon amino acid has been proposed as a causative factor in the amyotrophic lateral sclerosis-Parkinson dementia (ALS-PD) complex of Guam (6,18).

L-BMAA induces progressive motoneuronal damage and symptoms that closely resemble ALS in the cynomolgus monkey (19) and excitotoxic changes in rat and mouse cultured cortical neurons (9,14). The L-BMAA-induced neuronal damage is inhibited by competitive NMDA antagonists such as 2-amino-7-phosphonoheptanoic acid (AP-7) (14) and noncompetitive NMDA antagonists such as MK-801 (19). Therefore, L-BMAA may have excitatory effects on excitatory amino acid (EAA) receptors. EAA transmission has been proposed to be important in the genesis of seizures (8,10) and in other types of behavior such as "barrel rolling" (20). L-BMAA induces convulsions and hyperactivity in the monkey, chicken, mouse, and rat (11,15,19). Thus, it is of interest to investigate whether or not L-BMAA-induced behavioral changes relate to the excitation of EAA receptors.

In this study, we pharmacologically characterized L-BMAA-induced behaviors by pretreating rats with NMDA and non-NMDA EAA receptor antagonists. To achieve high concentrations of L-BMAA in the brain, ICV administration was utilized.

METHOD

Animals and Surgical Procedure

Male Sprague-Dawley rats (200–250 g) were housed under a 12 L : 12 D cycle condition and fed ad lib. They were anesthetized with ketamine HCl (115 mg/kg) and xylazine (4.6 mg/kg) IM. A 5.4 × 0.61-mm diameter polyethylene cannula (PE-10 7401: Clay Adams, Parsippany, NJ) was implanted into the right lateral ventricle using stereotaxic coordinates of AP −1.0 mm, lateral 1.5 mm, and ventral 3.5 mm from bregma according to the method of Hallak and Giacobini (2). Rats were allowed to recover at least 4 days. The number of rats used in each group is indicated in the result tables.

Chemicals

Highly purified L-BMAA HCl was custom synthesized by Research Biochemals, Inc. (Natick, MA). DL(±)2-Amino-5-phosphonopentanoic acid (AP-5) was obtained from Sigma

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Chemical Co. (St. Louis, MO) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) was purchased from Tocris Neuramin (Essex, England). [(+)5-Methyl]-10,11-dibenzo(*a,d*)-cycloheptan-5,10-imine maleate (MK-801) was a gift from Merck Sharp & Dohme Research Lab. (Rahway, NJ).

Drug Administration and Observation of Symptoms

L-BMAA was dissolved in 20 μl vehicle (25 mM $NaHCO_3$). The EAA antagonists were dissolved in 15 μl normal saline. L-BMAA or EAA antagonists were infused into the lateral ventricle over 10 s followed by a 5-μl saline infusion. Using a modification of the method of Irwin (4), we observed various behaviors including forepaw grooming, hindpaw grooming, chewing, eating, hyperactivity, vocalization, wet-dog shaking, splay, head swaying, facial tremor, tooth-chattering, abnormal gait, clonic convulsion, rigidity, sedation, breathing amplitude, and irritability. Splay was defined as a flat posture with spreading of all limbs. Clonic convulsion consisted of foreleg movements and quick and repetitive spreading of hindlegs behind the root of the tail. Rigidity consisted of a stiff flexed posture with lack of tactile responsiveness. These behavioral changes were scored during the following intervals: 0–30 s, 30 s–1 min, 1–2 min, 2–3 min, 3–4 min, 4–5 min, 5–7 min, 7–9 min, 9–11 min, 11–13 min, and 13–15 min. The scoring scale consisted of four levels: no change, mild (possibly change), moderate, and severe. The percentage of animals showing each behavior was calculated from the equation: (number of rats showing moderate and severe behaviors/total rats tested) × 100. Duration of clonic convulsions, splay, and rigidity were recorded. All observations were performed between 8:00 a.m. and 2:00 p.m. by an observer blinded to treatment. The position of the cannula in the lateral ventricle was verified by postmortem dissection.

Acute Experiments

In preliminary dose-response tests, an ICV injection of 1,000 μg L-BMAA induced severe splay and clonic convulsions followed by death. Two hundred fifty micrograms L-

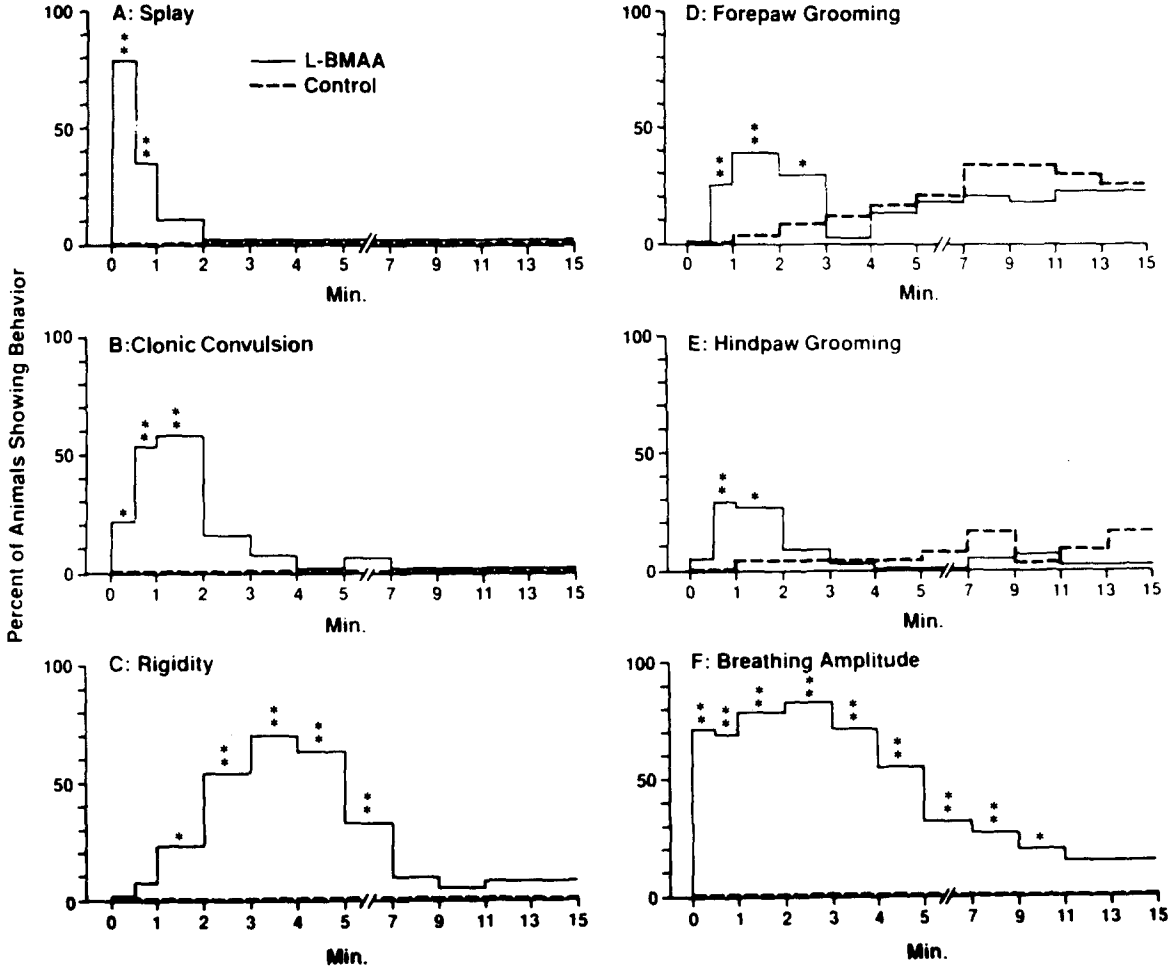

FIG. 1. Time course of behavioral changes induced by single ICV injection of L-β-methylamino-alanine (L-BMAA). The vertical axis indicates the percentage of animals showing behavior such as splay (A), clonic convulsion (B), rigidity (C), forepaw grooming (D), hindpaw grooming (E), and an increment of amplitude of breathing (F). The test group is indicated by a solid line and was injected with 500 μg L-BMAA ICV, and the control group is indicated by a dotted line and was treated as well as L-BMAA group (not injected). Statistically significant difference from corresponding control by χ^2 test. $^{**}p < 0.01$, $^{*}p < 0.05$, $^{+}p < 0.1$.

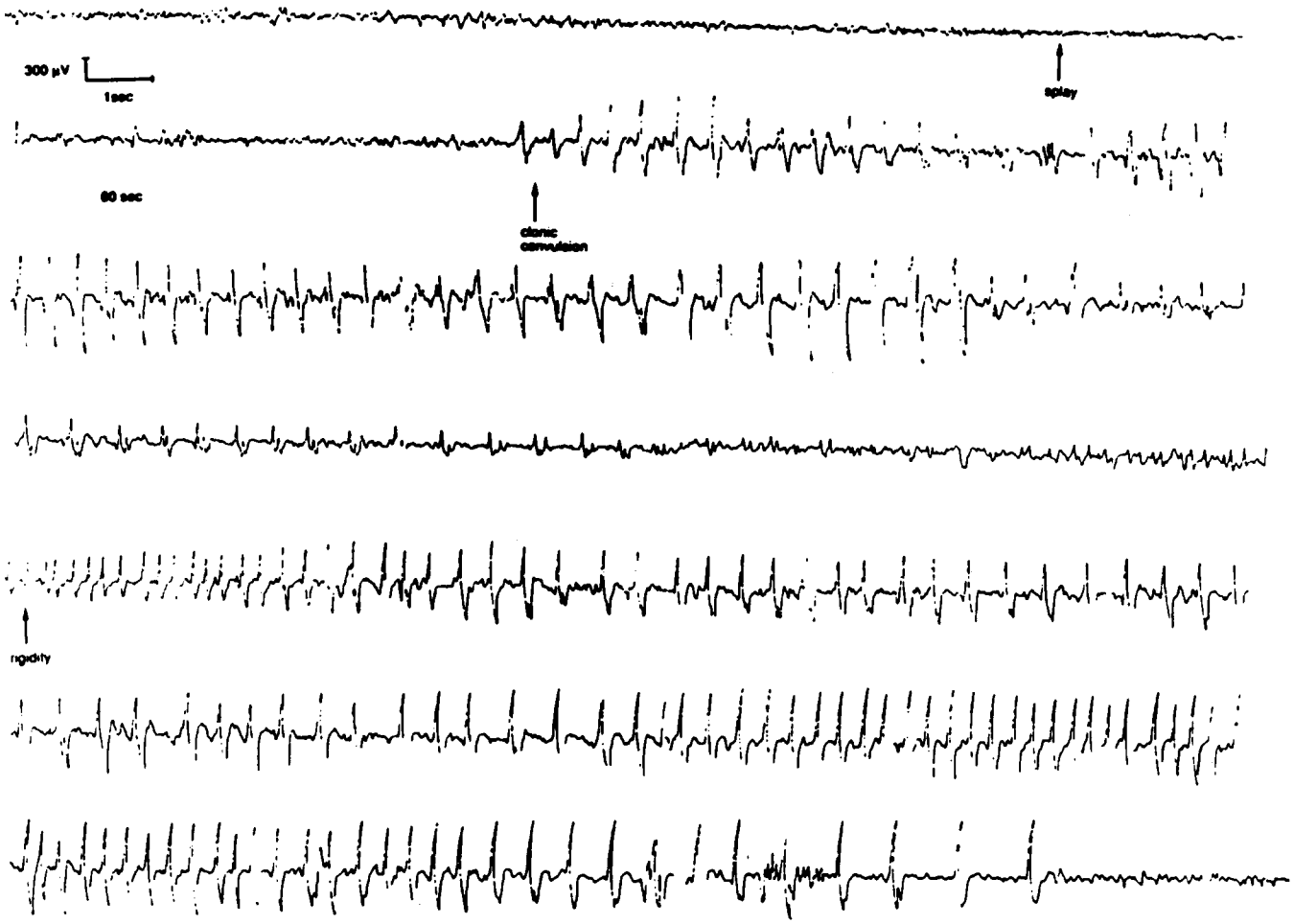

FIG. 2. Cortical electroencephalograph (EEG) recording taken from rat after ICV infusion of L-β-methylamino-alanine (L-BMAA). Recording performed using bipolar montage from frontal to parietal regions. Note epileptiform activity that occurs during clonus and during rigid posturing.

BMAA produced hyperactivity. The 500-μg dose induced several behavioral changes but was not lethal. This dose was selected for our experiments.

Five hundred micrograms L-BMAA was injected ICV and behavioral changes were observed during the following 15-min period. Controls were subjected to sham surgery but did not receive vehicle ($NaHCO_3$ solution).

Subacute Experiments

Single doses of 500 μg L-BMAA were injected ICV daily for 10 days. Control rats were injected with 20 μl vehicle (25 mM $NaHCO_3$). Behaviors were observed daily for 10 min after each injection.

Cross-over Studies

To choose the appropriate injection time and dose of EAA antagonists, we observed the behaviors produced by single injections of MK-801, AP-5, and DNQX. The doses selected for a following cross-over test were 5, 50, and 100 μg for MK-801, 10 and 50 μg for AP-5, and 10 and 20 μg for DNQX.

Rats were injected 500 μg L-BMAA and only those animals that demonstrated clonic convulsions within 15 min after L-BMAA injection were used. Two or 3 days later, they were divided into two groups and a cross-over study was performed. The first group was pretreated with one of the EAA antagonists. Five hundred micrograms L-BMAA were injected 15 min after ICV administration of either MK-801 or AP-5 or 10 min after DNQX. The second group was pretreated with 20 μl saline ICV at 15 or 10 min prior to ICV injection of 500 μg L-BMAA. Behaviors were observed for 15 min by a blinded observer. Two to 3 days after the first dosage of the cross-over test, the first group was pretreated with saline and the second group with EAA antagonists prior to L-BMAA infusion.

Electroencephalograph Study

Simultaneous recording of electroencephalograph (EEG) and behavior were performed to correlate the L-BMAA-induced behaviors with the EEG. Three animals were used for this study. Intraventricular cannulae were implanted as

TABLE 1

EFFECTS OF ICV INJECTION OF 20 μg DNQX ON L-BMAA (500 μg, ICV)-INDUCED BEHAVIORAL CHANGES

L-BMAA-Induced Behavior	Pretreatment	*n*	Mean ± SE
Splay (duration, min)	Saline	12	33.9 ± 6.9
	DNQX 20 μg	12	53.5 ± 10.8
Clonic convulsion (duration, seconds)	Saline	12	31.9 ± 4.5
	DNQX 20 μg	12	5.5 ± 3.2*
Clonic convulsion (latency, seconds)	Saline	11	59.5 ± 8.8
	DNQX 20 μg	6	127.2 ± 20.9*
Rigidity (duration, min)	Saline	12	2.63 ± 0.53
	DNQX 20 μg	12	0.42 ± 0.23*

			Percent of Animals Showing†	
L-BMAA-Induced Behavior	Pretreatment	*n*	Stronger Than Moderate Behavior	No Response
Splay (appearance)	Saline	12	75	8
	DNQX 20 μg	12	92	8
Clonic convulsion (appearance)	Saline	12	58	8
	DNQX 20 μg	12	0	50*
Rigidity (appearance)	Saline	12	67	17
	DNQX 20 μg	12	25	75‡

L-BMAA was injected ICV 10 min after infusion of DNQX or saline. After L-BMAA infusion, behavior was observed for 15 min.

*$p < 0.01$, ‡$p < 0.05$, significant difference from corresponding control.

†The values indicate the percentages of animals showing stronger than moderate behavior or no response in animals used.

described above. Stainless steel bone screws were implanted into the skull bilaterally over frontal and parietal cortex and connected to an Amphenol connector. Immediately after 500 μg L-BMAA infusion, behavior and EEG were simultaneously recorded using split video display techniques.

Statistical Analysis

Results are expressed as the mean ± SE. Statistical significance were assessed by Student's *t*-test and χ^2 test. The difference was considered significant at the $p < 0.05$ level.

RESULTS

Effects of Single Injection of L-BMAA

A single injection of 500 μg L-BMAA induced splay in 66% of rats ($n = 43$) for 30.4 ± 4.1 s, clonic convulsions in 49% rats for 25.9 ± 3.6 s, and rigidity in 73% rats for 3.21 ± 0.35 min in a sequence. Sham controls ($n = 23$) did not exhibit these behaviors (Fig. 1). L-BMAA induced irritability between periods of clonic convulsions and rigidity in 53% rats. The percentage of rats showing this behavior was significantly higher than control (0%, $p < 0.01$). Forepaw grooming appeared in 54%, hindpaw grooming in 49%, and wet-dog shaking in 47%. These behaviors occurred often together with clonic convulsions. Their appearance was statistically different from controls [forepaw grooming, in 13% of control ($p < 0.01$); hindpaw grooming, 9% ($p < 0.01$); wet-dog shaking, 17% ($p < 0.05$)] (Fig. 1.). L-BMAA also induced deep breathing in 88% of rats (different from controls in 0%, $p < 0.01$) starting immediately after treatment and lasting for 7 min. Following a period of rigidity, a phase of hyperactivity lasting about 1 min occurred in 91%, followed by wet-dog shaking (47%) and facial tremor (61%), which were significantly increased over control rats (22%, $p < 0.05$; 9%, $p < 0.01$, respectively). There were no significant differences in the percentages of rats showing chewing, forepaw and hindpaw grooming, or hyperactivity between controls and L-BMAA-treated rats during the period 5–15 min after injection.

Effect of L-BMAA on EEG

Facial-forelimb clonic seizures were accompanied by bilateral spike-wave activity on EEG (Fig. 2). At the end of the convulsive activity, the animal became rigid and remained in a motionless, arched, reared-back position. During the rigidity, high-voltage-spike-wave activity appeared and gradually declined and was followed by a general suppression of EEG activity, indicating that this rigid phase was also an ictal phenomenon. The behavior of excessive grooming was not accompanied by epileptiform EEG changes.

Subacute Effects of L-BMAA

In about 60% of rats, daily infusion of L-BMAA over the 10-day course induced behaviors similar to those seen after initial infusion. However, clonic convulsion, splay, rigidity, and an increased amplitude of breathing diminished at 4 days after beginning of administrations. Other behaviors (wet-dog

TABLE 2

EFFECTS OF ICV INJECTION OF 50 μg AP-5 ON L-BMAA (500 μg, ICV)-INDUCED BEHAVIORAL CHANGES

L-BMAA-Induced Behavior	Pretreatment	*n*	Mean ± SE
Splay (duration, min)	Saline	12	44.1 ± 6.9
	AP-5 50 μg	12	57.2 ± 6.6
Clonic convulsion (duration, seconds)	Saline	12	35.0 ± 5.7
	AP-5 50 μg	12	52.8 ± 13.7
Clonic convulsion (latency, seconds)	Saline	12	55.8 ± 8.9
	AP-5 50 μg	12	103.5 ± 18.1*
Rigidity (duration, min)	Saline	12	3.42 ± 0.68
	AP-5 50 μg	12	0.58 ± 0.58†

			Percent of Animals Showing‡	
L-BMAA-Induced Behavior	Pretreatment	*n*	Stronger Than Moderate Behavior	No Response
Splay (appearance)	Saline	12	67	0
	AP-5 50 μg	12	75	0
Clonic convulsion (appearance)	Saline	12	50	0
	AP-5 50 μg	12	42	17
Rigidity (appearance)	Saline	12	75	8
	AP-5 50 μg	12	8	92*

L-BMAA was injected ICV 10 min after infusion of AP-5 or saline. After L-BMAA infusion, behavior was observed for 15 min.

*$p < 0.05$, †$p < 0.01$, significant difference from corresponding control.

‡The values indicate the percentages of animals showing stronger than moderate behavior or no response in animals used.

shaking, forepaw and hindpaw grooming) were not different from the initial changes by day 6 of administrations. Ten percent of rats ($n = 31$) did not show clonic convulsions, 16% did not show splay, and 10% did not show rigidity within 10 days. Rats ($n = 11$) that showed clonic convulsions after the first injection, showed this symptom for 4.36 ± 0.53 times following single injections daily, splay for 5.09 ± 0.74 times, and rigidity for 4.36 ± 0.58 times. The remainder ($n = 20$) showed clonic convulsions for 2.00 ± 0.45 times, splay for 2.75 ± 0.70 times, and rigidity for 3.00 ± 0.56 times. There were statistical differences between responders and nonresponders (clonic convulsions, $p < 0.01$; splay, $p < 0.05$).

Effects of DNQX on L-BMAA-induced Behavioral Changes

Infusion of DNQX alone did not cause splay, clonic convulsion, or rigidity at doses up to 20 μg/rat. At doses higher than 10 μg, it induced ataxia, hyperactivity, facial tremor, chewing, and fore- and hindpaw grooming.

Pretreatment with DNQX 10 min prior to L-BMAA significantly decreased duration and severity of the clonic convulsions and the rigidity in a dose-dependent manner and prolonged latency of clonic convulsion onset (Table 1). DNQX failed to affect duration and severity of L-BMAA-induced splay. Pretreatment with 20 μg DNQX inhibited the forepaw and hindpaw grooming that occurred during a period of 3 min after L-BMAA injection ($p < 0.05$, respectively). DNQX (20 μg) decreased the wet-dog shaking ($p < 0.1$) and deep breathing ($p < 0.05$). L-BMAA-induced chewing was enhanced ($p < 0.01$), but the facial tremor was not affected by 20 μg DNQX.

Effects of AP-5 on L-BMAA-induced Behavioral Changes

Infusion of AP-5 alone at doses higher than 25 μg induced abnormal barrel rolling gait, chewing, and wet-dog shaking.

Pretreatment with the high dose of AP-5 (50 μg) 15 min prior to a 500-μg dose of L-BMAA prolonged latency of the clonic convulsion onset ($p < 0.05$) but failed to affect duration and severity of the clonic convulsion. L-BMAA-induced splay was also unaffected by AP-5. AP-5 significantly inhibited duration and severity of the L-BMAA-induced rigidity ($p < 0.01$, Table 2). Fifty micrograms AP-5 significantly inhibited the forepaw grooming ($p < 0.01$), tended to decrease the hindpaw grooming, and significantly enhanced the facial tremor ($p < 0.05$) induced by L-BMAA.

Effects of MK-801 on L-BMAA-induced Behavioral Changes

Doses higher than 20 μg MK-801 alone induced an abnormal barrel rolling gait, chewing, wet-dog shaking, facial tremor, forepaw grooming, slight hindpaw grooming, and slight decrease of breathing amplitude.

Pretreatment with 100 μg MK-801, 15 min prior to L-BMAA, completely blocked the rigidity ($p < 0.01$) but increased duration and severity of the splay ($p < 0.1$ and $p < 0.05$) (Table 3). On the other hand, MK-801 tended to enhance duration of L-BMAA-induced convulsion at 100 μg (Table 3: not statistically significant) and prolonged onset latency of L-BMAA-induced convulsion at 5 μg ($p < 0.05$) (Table 4). A 50-μg dose of MK-801 slightly increased duration of the clonic convulsion [74.6 ± 33.1 s in the L-BMAA plus MK-801-

TABLE 3

EFFECTS OF ICV INJECTION OF 100 μg MK-801 ON L-BMAA (500 μg, ICV)-INDUCED BEHAVIORAL CHANGES

L-BMAA-Induced Behavior	Pretreatment	n	Mean ± SE
Splay (duration, min)	Saline	8	18.5 ± 5.9
	MK-801 100 μg	8	37.8 ± 7.8*
Clonic convulsion (duration, seconds)	Saline	8	10.3 ± 7.4
	MK-801 100 μg	8	47.0 ± 20.6
Clonic convulsion (latency, seconds)	Saline	2	38.0 ± 8.0
	MK-801 100 μg	6	49.2 ± 11.1
Rigidity (duration, min)	Saline	8	3.75 ± 0.96
	MK-801 100 μg	8	0 ± 0†

			Percent of Animals Showing‡	
L-BMAA-Induced Behavior	Pretreatment	n	Stronger Than Moderate Behavior	No Response
Splay (appearance)	Saline	8	50	38
	MK-801 100 μg	8	88	0§
Clonic convulsion (appearance)	Saline	8	12	75
	MK-801 100 μg	8	25	38
Rigidity (appearance)	Saline	8	62	12
	ml-801 100 μg	8	0	100†

L-BMAA was injected ICV 10 min after infusion of MK-801 or saline. After L-BMAA infusion, behavior was observed for 15 min.

*$p < 0.1$, †$p < 0.01$, §$p < 0.05$, significant difference from corresponding control.

‡The values indicate the percentages of animals showing stronger than moderate behavior or no response in animals used.

treated group ($n = 5$) and 9.2 ± 9.2 s in the L-BMAA-treated group ($n = 5$) at $p < 0.1$]. One hundred micrograms MK-801 inhibited the forepaw grooming ($p < 0.01$) and tended to decrease the hindpaw grooming, facial tremor, and chewing that were induced by L-BMAA.

DISCUSSION

In our experiments, L-BMAA induced several behavioral changes summarized in Table 5. Polsky et al. (11) reported that IP injection of 840–1,680 μg/kg DL-BMAA induces convulsions in rats. Our results statistically confirmed their observations and showed that L-BMAA-induced clonic convulsions are accompanied by epileptiform EEG.

In our subacute experiments, these L-BMAA-induced behaviors were reproduced by repeated infusions of L-BMAA. Therefore, we could use cross-over tests to observe the effects of EAA antagonists on L-BMAA-induced behaviors. In these cross-over experiments, NMDA antagonists (MK-801 and AP-5) failed to inhibit L-BMAA-induced clonic convulsions but delayed the initiation of the convulsion, and a non-NMDA antagonist (CNQX) blocked L-BMAA-induced clonic convulsions. Lee and Hablitz (7) reported that non-NMDA EAA receptor antagonists such as CNQX are capable of markedly reducing picrotoxin-induced epileptiform activity. K. Sato et al. (17) reported that MK-801 does not elevate the threshold of seizure generation but delays the initiation of epileptic activity. Hirano and Hagiwara (3) suggested that non-NMDA receptor channels are located in the postsynaptic membrane while both NMDA- and non-NMDA receptor channels are present in the presynaptic membranes at the synapse between cerebellar granules and Purkinje cells. T. Sato et al. (16) found that intraamygdalar injections of combined glutamate/aspartate produce a strong excitation and seizures. These observations suggest that non-NMDA EAA receptors may play an important role in the expression of L-BMAA-induced clonic convulsions and NMDA receptors may be related to the regulation of the clonic convulsions.

Because L-BMAA-induced splay was not inhibited by AP-5, MK-801, or DNQX, this behavior may be mediated by other neurotransmitter receptors or another EAA receptor subtype. L-BMAA-induced rigidity was different from that induced by morphine or reserpine. Our EEG recording suggests that this rigidity likely represents an epileptic phenomenon rather than a catatonic state. MK-801 completely inhibited L-BMAA-induced rigidity, suggesting that this behavior may be mediated by NMDA receptors. Although both MK-801 and AP-5 act at the NMDA binding receptor complex, Kavanaugh et al. (5) reported that MK-801 also affects the channel associated with nicotinic receptors. This may explain the slight differences of effect of MK-801 and AP-5 on L-BMAA-induced behaviors.

Our results indicate that L-BMAA may act upon both NMDA and non-NMDA receptors. Ross et al. (14) and Spencer et al. (19) reported that BMAA-induced cellular changes were selectively antagonized by AP-7. AP-5 also reduced a transitory hyperexcitatory state that was induced by a single

TABLE 4

EFFECTS OF ICV INJECTION OF 5 μg MK-801 ON L-BMAA (500 μg, ICV)-INDUCED BEHAVIORAL CHANGES

L-BMAA-Induced Behavior	Pretreatment	*n*	Mean ± SE
Splay (duration, min)	Saline	8	37.1 ± 3.3
	MK-801 5 μg	8	38.8 ± 7.5
Clonic convulsion (duration, seconds)	Saline	8	35.4 ± 11.1
	MK-801 5 μg	8	53.6 ± 13.8
Clonic convulsion (latency, seconds)	Saline	6	32.8 ± 4.5
	MK-801 5 μg	6	48.3 ± 4.8*
Rigidity (duration, min)	Saline	8	1.50 ± 0.80
	MK-801 5 μg	8	0.13 ± 0.13

			Percent of Animals Showing†	
L-BMAA-Induced Behavior	Pretreatment	*n*	Stronger Than Moderate Behavior	No Response
Splay (appearance)	Saline	8	62	0
	MK-801 5 μg	8	62	12
Clonic convulsion (appearance)	Saline	8	62	25
	MK-801 5 μg	8	75	25
Rigidity (appearance)	Saline	8	38	62
	MK-801 5 μg	8	0	88

L-BMAA was injected ICV 10 min after infusion of MK-801 or saline. After L-BMAA infusion, behavior was observed for 15 min.

*$p < 0.05$, significant difference from corresponding control.

†The values indicate the percentages of animals showing stronger than moderate behavior or no response in animals used.

TABLE 5

EFFECTS OF EXCITATORY AMINO ACID ANTAGONISTS ON L-BMAA-INDUCED BEHAVIOR

	spl.	clon. conv.	rigd.	fore grmn.	hind grmn.	chew	wet dog	face trem.	deep brth.
L-BMAA (500 μg)	+	+	+	+	+	−	+	(+)	+
DNQX + L-BMAA									
20 μg	±	↓	↓	↓	↓	↑	(↓)	±	↓
10 μg	±	(↓)	±	±	(↓)	↑	±	±	(↓)
DNQX alone	−	−	−	+	+	+	(+)	(+)	(+)
MK-801 + L-BMAA									
100 μg	↑	↑	↓	↓	(↓)	(↓)	±	↓	±
50 μg	↑	↑	↓	↓	±	(↓)	±	(↓)	±
5 μg	±	(↑)	↓	±	±	±	±	(↓)	±
MK-801 along	−	−	−	+	−	+	+	+	R
AP-5 + L-BMAA									
50 μg	±	↑	↓	↓	±	±	±	↑	±
10 μg	±	↑	(↓)	(↓)	±	±	±	±	±
AP-5 alone	−	−	−	−	−	+	+	−	(+)

spl., splay; clon. conv., clonic convulsion; rigd., rigidity; fore grmn., forepaw grooming; hind grmn., hindpaw grooming; chew., chewing; wet dog, wet-dog shaking; face trem., facial tremor; deep brth., increase in breathing amplitude; +, significant induction of behavior; (+), tendency of behavior to induce; −, no behavior; R, reduced breathing amplitude; ↑, significant enhancement of L-BMAA-induced behavior; (↑), tendency of enhancement of L-BMAA-induced behavior; ↓, significant reduction of L-BMAA-induced behavior; (↓), tendency of reduction of L-BMAA-induced behavior; ±, no change.

dose of 1,000 μg ICV L-BMAA (15). Weiss and Choi (21) found that L-BMAA-induced damage in NMDA diaphorase cells is more effectively antagonized by kynurenate than AP-7. Richter and Mena (13) found that in the presence of high (100 μM) concentrations of NMDA L-BMAA reduced L-glutamate biding and suggested that L-BMAA's action may not be exclusively at NMDA receptors. In a parallel neurochemical study (12), we found that L-BMAA occupied both glutamate and AMPA binding sites in vitro in the presence of bicarbonate. Further, Copani et al. (1) reported that L-BMAA acts as a mixed agonist of "metabotropic" and NMDA receptors. We could conclude that L-BMAA may act upon several kinds of EAA receptors such as NMDA, non-NMDA, and other EAA receptors and cause pronounced behavioral changes.

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