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Effect of Lidocaine on the Release, Receptor Binding and Uptake of Amino Acid Neurotransmitters *In vitro*

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ABSTRACT

The author examined the effects of lidocaine on the veratrine-or potassium-induced release of neurotransmitters to determine the possible role of amino acid neurotransmitters in lidocaine-induced convulsion.

The examined transmitters were gamma-aminobutyric acid (GABA), aspartic acid, glutamic acid and norepinephrine which are released from the synaptosomes. Furthermore, the effects of lidocaine on the binding to receptors and synaptosomal uptake of the two transmitters, GABA and glutamic acid, were determined in crude synaptic membranes and synaptosomes. In addition, the effects of propranolol, norepinephrine and serotonin on the release of amino acid neurotransmitters were also examined.

The veratrine-induced release of GABA was most severely inhibited by lidocaine and propranolol, while norepinephrine and serotonin reduced the release of aspartic acid and glutamic acid more than the GABA release. Generally the potassium-induced release was much more resistant to the lidocaine action than the veratrine-induced release. Among the neurotransmitters examined, the aspartic acid release was most prone to the lidocaine action, while the GABA release was most resistant.

Concentrations of lidocaine below 1 mM did not significantly change the GABA and glutamic acid receptor binding and uptake.

These results indicate that the blocking of sodium channels by lidocaine can result in the selective depression of the GABA release. This may result in unlimited excitation of the central nervous system.

Key Words: Lidocaine; Neurotransmitters; Synaptosome: Convulsion
Abbreviations: ASP, aspartic acid; GABA, gamma-aminobutyric acid; GLU, glutamic acid; IC, inhibitory concentration.

INTRODUCTION

Lidocaine and other local anesthetics stabilize membranes largely by blocking sodium channels. When given systemically, these anesthetics may prompt various toxic responses in the central nervous system (CNS), including sedation, hypnosis or convulsion. These effects will vary according to blood level(Koppanyi, 1962; Moore and Brdienbaugh, 1960; Munson *et al.*, 1970). But the mechanism of these apparently opposite effects of local anesthetics on the CNS (i.e. sedative, anticonvulsant action versus convulsant action), cannot be explained clearly. It is believed that the action of local anesthetics on the CNS neurons act as depressants in the same way they would in the peripheral nervous system. Through electrophysiological studies, some investigators have suggested that a low concentration of lidocaine depress the inhibitory synaptic transmission more efficiently than the excitatory synaptic transmission which results in CNS excitation (de Jong *et*

al., 1969; Tanaka and Yamasaki, 1966). Although experimental evidence is not conclusive, this concept has been widely accepted.

Several species of neurotransmitters are involved in synaptic transmission. Some investigators have suggested that in a local anesthetic-induced convulsion, changes in the noradrenergic pathway might be responsible for this toxic effect (Oh and Cheong, 1985), while others have suggested that the dopaminergic (Ciarlone, 1981; Ciarlone and Smudski, 1977) or serotonergic pathway (Ciarlone and Smudski, 1976; Ciarlone and Smudski, 1977; Ciarlone, 1981; Ciarlone and Juras, 1981; Kim and Cheong, 1987; Niederlehner *et al.*, 1982) might be responsible. The results, however, were equivocal for any pathway.

Many amino acids are also known to function as neurotransmitters in the mammalian CNS (Cooper *et al.*, 1986). The most prevalent inhibitory neurotransmitter is GABA, while aspartic acid and glutamic acid are excitatory neurotransmitters in various sites within the CNS. Among these, the GABAergic pathway is the target of many convulsant and anticonvulsant drug actions (Jobe and Laird, 1981; Laursen, 1984; Mandel *et al.*, 1981; Schousboe *et al.*, 1983). The GABAergic receptor blockade resulted in convulsions, but the augmentation of the GABA level in some sites protected animals used in experiments from generalized convulsions (Iadarola and Gale, 1982; Jobe and Laird, 1981; Kendall *et al.*, 1981). Previously, Ikeda *et al.* (1983) reported the inhibitory effect of several local anesthetics on the release of exogenously labeled GABA.

Most of the studies on the mechanism of the local anesthetic-induced convulsion have employed *in vivo* biochemical analysis. But the release, receptor binding or uptake of neurotransmitters can be studied more precisely by *in vitro* experiments. Brain slices or synaptosomal preparations of rats have been used for this purpose.

In order to discover a possible mechanism of the lidocaine-induced convulsions, the present study examines the differential effect of lidocaine on: 1) the endogenous release of GABA, aspartic acid, glutamic acid and norepinephrine from synaptosomal preparations: 2) GABA and glutamic acid receptor binding: and 3) the synaptosomal uptake of GABA and glutamic acid.

MATERIALS AND METHODS

Materials

[^{3}H]-GABA and [^{3}H]-glutamic acid were purchased from the New England Nuclear Co. (Boston, MA., USA). Lidocaine, propranolol HCl, norepinephrine HCl, 5-hydroxytryptamine creatinine sulfate complex (serotonin) and veratrine HCl (a mixture of cevadilline, sabadine, cevadine and veratridine) were purchased from the Sigma Chemical Company (St. Louis, Missouri, USA). The inorganic salts and glucose contained in the incubation medium were analytical grade and were purchased from Merck Compnay (W. Germany).

Synaptosome preparation

Female Sprague-Dawley rats weighing around 200 g were used throughout these experiments. A synaptosomal fraction was prepared from the whole brain (excluding the cerebellum) using the method described by the Dodd *et al.* (1981) The rats were sacrificed by cervical dislocation, and the brains were homogenized in 10 volumes of ice-cold, 0.32 M sucrose solution buffered with 5 mM tris-HCl (pH 7.2).

Homogenate was centrifuged at 1,000$\times$g for 10 minutes. The supernatant was then collected, layered on a 1.2 M sucrose solution and centrifuged at 160,000$\times$g for 22 minutes (w^2t setting$=1.83\times10^{10}$ rad^2/sec). After centrifugation, the interface was collected and diluted with the same volume of 0.32 M sucrose solution. The diluted interface fraction was layered on 0.8 M sucrose solution and again centrifuged at 160,000$\times$g for 22 minutes. A synaptosomal fraction was obtained from the pellet.

Synaptosomal release of amino acids and norepinephrine

The synaptosomal pellet was lightly rinsed with ice-cold, Krebs-bicarbonate solution(NaCl 118 mM, KCl 4.75 mM, $CaCl_2$ 2.52 mM, KH_2PO_4 1.18 mM, $MgSO_4$ 1.19 mM, $NaHCO_3$ 25 mM, glucose 10 mM) and resuspended in the same solution, which was previously aerated with 95% O_2/5% CO_2 for 30 minutes and adjusted to pH 7.4. One and six tenth ml aliquots of synaptosomal suspension (2.5–3 mg protein/assay) were incubated at 37℃ for 30 minutes in shaking water bath.

Various concentration of lidocaine, propranolol, norepinephrine or serotonin was then added, as indicated in tables and figures. Ten minutes after adding lidocaine or propranolol (one minute in the case of norepinephrine or serotonin), the synaptosomes were depolarized with veratrine (final conc. 20μM) or KCl (final K^+ conc. 50 mM). After veratrine was added (five minutes in the case of potassium stimulation), the incubation was continued for an additional 10 minutes and terminated by centrifuging at 25,000×g for five minutes.

The supernatant was collected, freeze-dried and redissolved in 30 mM $LiCO_3$ buffer(final pH 9.5), dansylated using the method described by Tapuhi *et al.* (1981) and analyzed using high performance liquid chromatography (HPLC).

In some experiments, several brain regions were dissected, and synaptosomal fractions were obtained to analyze amino acid release in a particular brain site. Striatum and hippocampus were dissected using the method of Glowinski and Iversen (1966). The ventral mid-brain including the nucleus substantia nigra was dissected following the rat brain atlas (König *et al.*, 1974). For the synaptosomal release of norepinephrine, all the other procedures were identical to the amino acid release experiment except the incubation medium which contained ascorbic acid (290μM) and disodium EDTA (29μM) as antioxidants and 1μM desipramine as the uptake inhibitor.

Chromatographic condition for amino acid and norepinephrine analysis

Amino acids were analyzed by the Waters HPLC system, consisting of the Model 6000A solvent delivery system, the U6K injector, the M440 absorbance detector and the M730 data module. The hibar RP-8 column(Merck Co., W. Germany) was used in the stationary phase, and the 30 mM phosphate buffer was used in the mobile phase. A pH 6.9 mobile solution was used for the acidic amino acid analysis, while a pH 3.0 mobile solution was used for the GABA analysis. Acetonitrile was used as organic modifier at concentrations of 10 to 50 percent. Absorbance was measured at 254 nm. Norepinephrine was analyzed after extraction by the alumina adsorption method. The stationary phase was a Waters Radial-PAK μ Bondapak C18 column, and the mobile solution was pH 3.9, 0.15 M monochloroacetate buffer containing citric acid (0.03 M), disodium EDTA (2 mM) and sodium octylsulfate (100 mg/L). The eluted norepinephrine was detected voltametrically at 640 mV with the amperometric detector (Bioanalytical system). The remaining components of the HPLC system were the same as those used in the amino acid analysis.

Crude synaptic membrane preparation

The crude synaptic membrane was prepared from the whole brain excluding the cerebellum, using the method described by Zukin *et al.*(1974).

The brain was homogenized in 15 volumes of ice-cold, 0.32 M sucrose solution and centrifuged at 1,000×g for 10 minutes. The supernatant was collected and recentrifuged at 20,000×g for 20 minutes. After decanting the supernatant, the pellet was sonicated in 50 volumes of deionized water and centrifuged at 8,000×g for 20 minutes. The supernatant and buffy upper coat were carefully collected and centrifuged at 40,000×g for 20 minutes. The resultant pellet was stored at -30℃ for at least 24 hours to achieve the GABA binding assay. For the glutamic acid receptor binding assay, the resultant pellet was washed once with deionized water and once with a sodium free tris-acetate buffer (pH 7.4) by repeating resuspension and centrifugation at 40,000×g for 20 minutes. Then the pellet was resuspended in an 0.32 M sucrose solution and stored at −80℃ until used.

GABA receptor binding assay

The GABA receptor binding assay was performed using the method of Horton *et al.*(1982). The frozen crude synaptic membrane was thawed by adding deionized water and centrifuged at 40,000×g for 20 minutes. The pellet was again washed with sodium-free tris-citrate buffer (50 mM, pH 7.1). The synaptosomal membrane suspension was distributed in assay tubes to contain 0.4-0.5 mg protein in a volume of 1 ml. The incubation was carried out at 0℃ for 10 minutes after adding [^{3}H]-GABA (final conc. 10 nM) and terminated by centrifuging at 40,000×g for 10 minutes. After decanting the supernatant, the pellet was washed with the ice-cold incubation medium. Any remaining medium was removed with cotton buds. The pellet was solubilized with Protosol (New England Nuclear), and Liquifluor (New England Nuclear) solution was added. After 24 hours, radioactivity was determined by liquid

scintillation counting.

To determine non-specific binding, the binding assay was carried out in a medium containing additional non-labelled GABA (1 mM). To obtain specific binding, this value was subtracted from the total count of all samples.

Glutamic acid receptor binding assay

The glutamic acid binding assay was performed using the Ogita and Yoneda method(1986). The frozen crude synaptic membrane was thawed and centrifuged at 40,000×g for 40 minutes. After decanting the supernatant, the pellet was washed with 50 mM tris-acetate buffer(pH 7.4), then resuspended in the same buffer. The synaptosomal membrane suspension was distributed in the assay tubes to contain 0.4–0.5 mg protein in 1 ml volume. The membrane suspension was then incubated at 30℃ for 1 hr after the addition of [^{3}H]-glutamic acid (final conc. 20 nM). After the incubation, the samples were centrifuged at 40,000×g for 10 minutes. The pellet was washed with the ice-cold incubation medium, then processed as in the GABA binding assay.

To determine non-specific binding, the binding assay was carried out in a medium containing 1 mM non-labelled glutamic acid. Specific binding was calculated as the GABA receptor binding assay.

GABA and glutamic acid uptake experiment

The synaptosomal uptake experiment was carried out using the method described by Poli *et al.* (1985), with some modification. For example, the incubation medium was a Krebs-Ringer bicarbonate solution composed of NaCl 124 mM, KCl 3.5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.3 mM, $CaCl_2$ 1.2 mM, $NaHCO_3$ 25 mM and glucose 10 mM. The synaptosomal suspensions were distributed to assay tubes to contain 0.6–0.7 mg protein and preincubated for 30 minutes at 37℃. Then the [^{3}H]-GABA/non-labelled GABA mixture (final conc. 1μM) or [^{3}H]-glutamic acid/non-labelled glutamic acid mixture (final conc. 1μM) was added and incubated for five minutes. The incubation was terminated by centrifuging at 25,000×g for five minutes at room temperature. The pellet was washed with an incubation medium, and the tube was dried. As in the binding assay, radioactivity was determined by liquid scintillation counting.

The diffusional blank value was measured by incubation synaptosomal suspensions at 0℃, and this value was subtracted from the total count of each sample.

Protein determination

Aliquots from all samples were sonicated in a small amount of 0.2 N NaOH, and the protein was then determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

RESULTS

Effect of lidocaine on veratrine-induced amino acid release

The amount of amino acids released spontaneously was 93.6 nmol/100 mg protein, 199.3 nmol/100 mg protein and 42.7 nmol/100 mg protein for aspartic acid, glutamic acid and GABA, respectively. The release of amino acids was increased by adding 20μM of veratrine (Table 1). Since there is little conversion of glutamic acid to GABA in synaptosome, this increase seems to be solely due to its release. Among the three amino acids, the release of GABA was most prominently increased

Table 1. Effect of veratrine stimulation (20 μM) on amino acid release from whole brain synaptosomes

	Amino acid amount (nmol/100mg protein)		
	ASP	GLU	GABA
Spontaneous	93.6 ± 11.6	199.3 ± 21.8	42.7 ± 3.3
Stimulated	495.3 ± 53.5	1269.4 ± 102.1	693.9 ± 41.1
Folds of increase	5.3	6.4	16.3

Values are mean ± SE of 3–4 samples
ASP – aspartic acid, GLU – glutamic acid, GABA – gamma-aminobutyric acid

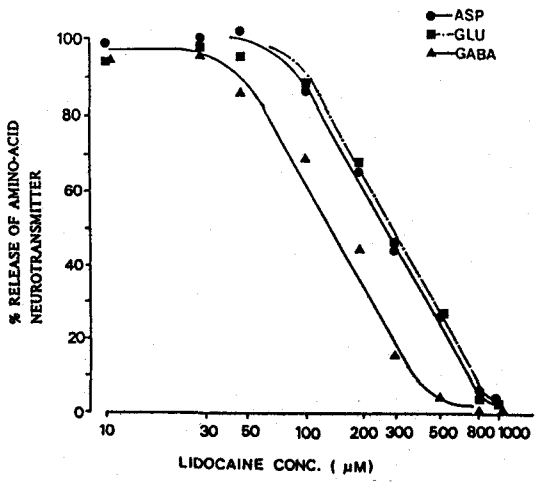

Fig. 1. Inhibitory effect of lidocaine on veratrine-induced release of amino acid neurotransmitters. % release indicates the percentage of net stimulated release of neurotransmitters to control assay calculated by subtracting spontaneous release.

Table 2. Inhibitory concentration 50 (IC_{50}) of lidocaine on veratrine-induced release of amino acids from whole brain synaptosomes

	ASP	GLU	GABA
IC_{50} (μM)	268	289	144
95% confidence limit (μM)	230–313	252–331	122–169

by veratrine. The release of amino acids was reduced by lidocaine above 100μM concentrations in a dose-dependent fashion. The release of GABA was reduced most markedly among the transmitters by lidocaine (Fig. 1). The concentrations of lidocaine necessary for 50 percent inhibition (IC_{50}) of the veratrine-induced release of aspartic acid, glutamic acid and GABA were 268μM, 289μM and 144μM respectively (Table 2).

Among the three brain regions examined, the spontaneous release of aspartic acid and glutamic acid was highest in the ventral mid-brain, and GABA release was highest in the striatum. However when stimulated by veratrine, the folds of increase in the amino acid release of aspartic acid, glutamic acid and GABA (Table 3) was highest in the hippocampus. The veratrine-induced release of the transmitters in those regions was also significantly reduced by lidocaine, and the reduction was similar to that examined in whole brain synaptosome. The differential sensitivity between the release of aspartic acid, glutamic acid and GABA was also observed as it was in the whole brain synaptosome. The ventral mid-brain seems to be most resistant to the lidocaine action (Table 4).

Effect of propranolol on veratrine-induced amino acid release

As shown in Fig. 2, the reduction of veratrine-induced amino acid release by propranolol was more significantly potent than that by lidocaine. Propranolol inhibited the veratrine-stimulated release of aspartic acid, glutamic acid and GABA: the 50 percent inhibition of the release of aspartic

Table 3. Effect of veratrine stimulation (20 μM) on amino acid release from synaptosomes of several brain regions

		Amino acid amount (nmol/mg protein)		
		ASP	GLU	GABA
Striatum	Spontaneous	111.7 ± 29.0	175.5 ± 31.8	56.4 ± 9.6
	Stimulated	623.7 ± 51.0	1080.4 ± 96.5	561.4 ± 27.7
	Folds of increase	5.6	6.2	10.0
Hippocampus	Spontaneous	42.1 ± 8.2	80.7 ± 11.2	24.0 ± 3.4
	Stimulated	718.6 ± 33.2	1073.4 ± 108.8	537.5 ± 25.0
	Folds of increase	17.1	13.3	22.4
Ventral midbrain	Spontaneous	374.3 ± 83.2	563.5 ± 73	52.6 ± 7.0
	Stimulated	642.0 ± 60.4	939.3 ± 85.8	739.5 ± 37.8
	Folds of increase	1.7	1.7	14.1

Values are mean ± SE of 4 samples.

Table 4. Inhibitory concentration 50 (IC_{50}) of lidocaine on veratrine-induced release of amino acids from synaptosomes of several brain regions

		ASP	GLU	GABA
Striatum	IC_{50} (μM)	213	262	173
	95% confidence limit (μM)	169–268	198–346	148–202
Hippocampus	IC_{50} (μM)	205	223	173
	95% confidence limit (μM)	178–237	180–276	150–201
Ventral midbrain	IC_{50} (μM)	197	304	197
	95% confidence limit (μM)	103–374	118–780	171–228

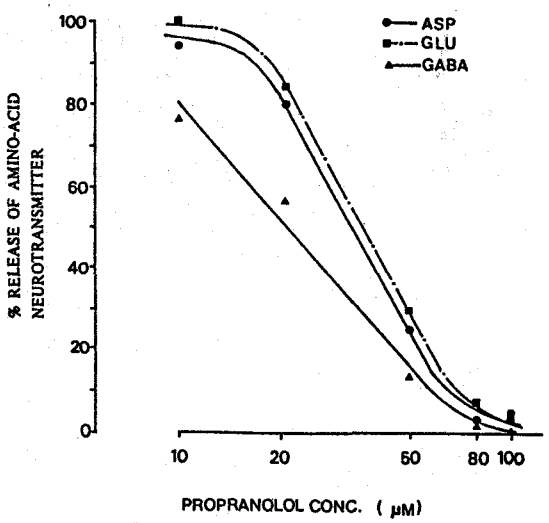

Fig. 2. Inhibitory effect of propranolol on veratrine-induced release of amino acid neurotransmitters. % release was calculated as in Fig. 1.

acid, glutamic acid and GABA was shown by 33, 36 and 21μM of propranolol, respectively (Table 5).

Effect of norepinephrine and serotonin on veratrine-induced amino acid release

No significant changes in the amino acid release were found at concentrations of 1μM or 10μM. At a concentration of 100μM the monoamines reduced the release of the amino acids in different fashion from lidocaine and propranolol. Norepinephrine reduced aspartic acid, glutamic acid and GABA release induced by veratrine to 45 percent, 56.7 percent and 60.3 percent, respectively.

Table 5. Inhibitory concentration 50 (IC_{50}) of propranolol on veratrine-induced release of amino acids from whole brain synaptosomes

	ASP	GLU	GABA
IC_{50} (μM)	33	36	21
95% confidence limit (μM)	26–42	29–46	16–28

Table 6. Effect of norepinephrine on veratrine-induced relaease of amino acids from whole brain synaptosomes

Norepinephrine concentration (μM)	% release		
	ASP	GLU	GABA
1	95.0 ± 6.4	101.7 ± 4.3	98.3 ± 2.8
10	95.0 ± 3.1	99.7 ± 0.7	95.0 ± 2.1
100	45.0 ± 3.0[a]	56.7 ± 3.3	60.3 ± 2.8

Values are mean ± SE of 3 samples
a : $P < 0.05$, Significantly different from GABA
% release was calculated as in Fig. 1.

Table 7. Effect of serotonin on veratrine-induced release of amino acids from whole brain synaptosomes

Serotonin concentration (μM)	% release		
	ASP	GLU	GABA
1	103.0 ± 0.6	102.0 ± 1.7	94.7 ± 3.6
10	89.7 ± 1.3	101.7 ± 2.4	94.3 ± 3.4
100	64.7 ± 8.1	81.0 ± 4.0	79.7 ± 3.8

Values are mean ± SE of 3 samples
% release was calculated as in Fig. 1.

Serotonin also reduced the amino acid release at a concentration of 100μM but the effect was less than that of norepinephrine (Tables 6,7).

Table 8. Effect of veratrine stimulation (20 uM) on norepinephrine release from whole brain synaptosomes

	Norepinephrine amount (pg/mg protein)
Spontaneous	473 ± 11
Stimulated	1200 ± 81
Folds of increase	2.5

Values are mean ± SE of 4 samples

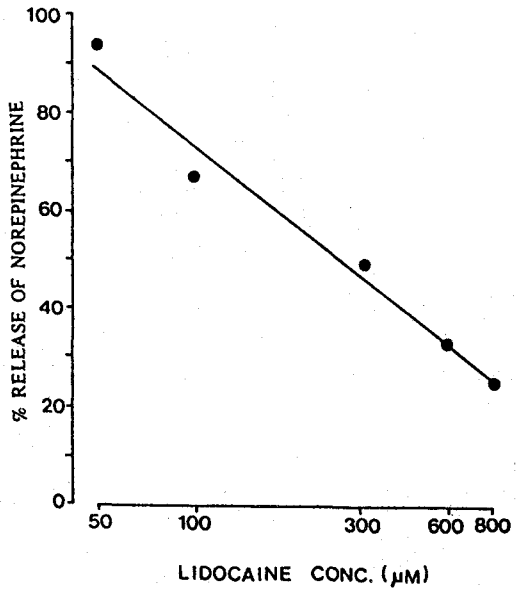

Fig. 3. Inhibitory effect of lidocaine on veratine-induced norepinephrine release.
% release was calculated as in Fig. 1.

Effect of lidocaine on veratrine-induced norepinephrine release

Veratrine significantly increased the release of norepinephrine from whole brain synaptosome (Table 8), while lidocaine reduced the release of norepinephrine. IC_{50} for the norepinephrine release was 272 μM (Fig. 3, Table 9).

Effect of lidocaine on potassium-induced amino acid release

Lidocaine reduced the potassium-induced release of the amino acids at higher concentration than necessary for the veratrine-induced release (Table 5,10,11). Also the patterns of sensitivity of the three amino acids to lidocaine were strikingly different from that observed in the veratrine-induced release. The GABA release was the most resistant and the aspartic acid release was the most sensitive (Fig. 4).

Effect of lidocaine on glutamic acid and GABA receptor binding

At 10 mM, both glutamic acid binding and GABA binding were reduced to 46 percent and 63 percent of the control value. But no significant changes in receptor binding were observed at concentrations below 1 mM (Fig. 5).

Table 9. Inhibitory concentration 50 (IC_{50}) of lidocaine on veratrine-induced norepinephrine release from whole brain synaptosomes

IC_{50} (μM)	95% confidence limit (μM)
272	211 – 350

Table 10. Effect of potassium stimulation (50 mM) on amino acid release from whole brain synaptosomes

	Amino acid amount (nmol/mg protein)		
	ASP	GLU	GABA
Spontaneous	103.9 ± 15.3	189.3 ± 26.1	80.9 ± 10.5
Potassium-stimulated	279.2 ± 38.1	719.8 ± 135.5	194.5 ± 44.0
Folds of increase	2.7	3.8	6.3

Values are mean ± SE of 4 samples

Table 11. Inhibitory concentration 50 (IC_{50}) of lidocaine on potassium stimulated release of amino acids from whole brain synaptosomes

	ASP	GLU	GABA
IC_{50} (mM)	1.10	1.85	4.31
95% confidence limit (mM)	0.94–1.29	1.51–2.28	2.43–7.66

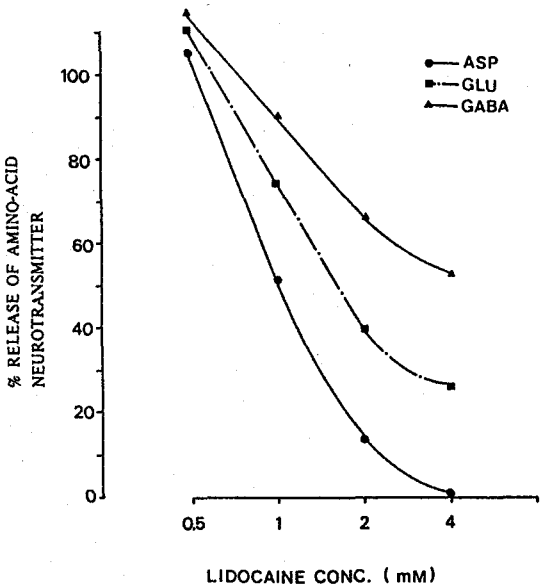

Fig. 4. Inhibitory effect of lidocaine on potassium-induced release of amino acid neurotransmitters. % release was calculated as in Fig. 1.

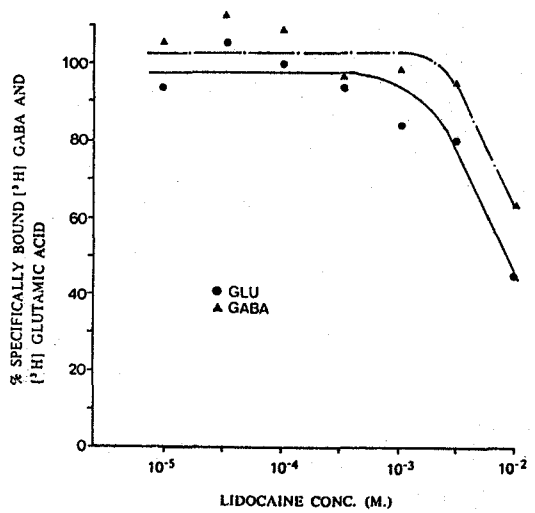

Fig. 5. Effect of lidocaine on GABA and glutamic acid receptor binding.

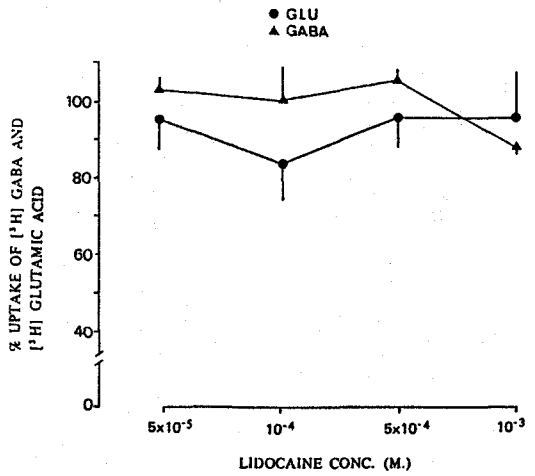

Fig. 6. Effect of lidocaine on synaptosomal GABA and glutamic acid uptake.

Effect of lidocaine on glutamic acid and GABA uptake

At concentrations of lidocaine used in this study (31.6 μM-1 mM), no apparent changes in the glutamic acid and GABA uptake were observed (Fig. 6).

DISCUSSION

Low doses of local anesthetic administered through intravenous injection produce a profound anticonvulsive and anesthetic effect. However in higher doses, they are known to induce convulsion (Munson *et al.*, 1970; Tanaka and Yamasaki, 1966; Usubiaga *et al.*, 1966; Wagman *et al.*, 1967; Wikinski *et al.*, 1970). Regarding the underlying mechanism of the local anesthetic-induced convulsions, de Jong and Wagman (1963)suggested a rhinencephalic focus (amygdala) for their convulsant effect. Tanaka and Yamasaki (1966) reported that lidocaine given intravenously selectively

blocked inhibitory synapses of cortical neurons. Similarly, Tuttle and Elliott (1969) and Usubiaga *et al.* (1966) have suggested that local anesthetics might block the inhibitory effects of the hippocampus and amygdala, i.e. effectively lower its threshold to olfactory inputs, and result in seizure activity. Later, de Jong *et al.* (1969) reported that spinal monosynaptic transmission was facilitated by lidocaine, probably by releasing the reflex arc from inhibitory control. They suggested that the convulsant action of lidocaine on the amygdala and hippocampus might simply reflect the generally enhanced excitability of central neurons due to the selective depression of inhibitory synaptic control by lidocaine.

Although the seizure mechanism is unknown, experimental evidence has shown not only that the brain seizure threshold can be altered by manipulation of 5-hydroxytryptamine (5-HT), norepinephrine, dopamine and other central neurotransmitters, but that these changes seem to depend on the experimental seizure model studied (Jobe and Laird, 1981).

As for the lidocaine-induced convulsions, the manipulation of monoamines also altered the seizure threshold. The serotonin depletion significantly lowered the seizure threshold for lidocaine in rats. The dopamine depletion also lowered the seizure threshold, but the depletion of norepinephrine did not affect seizure sensitivity (Ciarlone, 1981). Lidocaine stimulated the synthesis of 5-HT and reduced the release of dopamine but did not significantly change the norepinephrine release (Ciarlone and Smudski, 1977). Ciarlone and Smudski (1976) reported that lidocaine depleted central 5-HT and dopamine with no change in norepinephrine content in mice. But others reported that the depletion of serotonin protected rats from lidocaine convulsions and that norepinephrine and dopamine depletion have no effect on the seizure threshold (Niederlehner *et al.*, 1982). Furthermore, the augmentation of central 5-HT level by 5-hydroxytryptophan lowered the, seizure thresholds in mice, and the depletion of 5-HT increased the seizure thresholds for lidocaine (de Oliveria and Bretas, 1974).

However, there are few reports on the role of GABA in lidocaine convulsion. GABA is considered to be a major inhibitory neurotransmitter in manmmalian CNS. Compounds capable of activating GABAergic transmission may also be useful antiepileptic drugs. Furthermore, some GABA agonists are capable of inhibiting or attenuating a varietv of experimetal seizures. GABA receptor antagonists, such as bicuculline, invariably produced tonic-clonic convulsions in animals (Schousboe *et al.*, 1983). Recently Ikeda *et al.* (1983) reported that lidocaine and other local anesthetics block the stimulated release of exogenously labeled GABA.

The inhibition of veratrine-induced GABA release by lidocaine was more significantly susceptible than that of glutamiç acid and aspartic acid: the IC_{50} of lidocaine for GABA release was 144μM, while the IC_{50} of lidocaine for glutamic acid and aspartic acid were 289μM and 268μM, respectively (Fig. 1 and Table 2). Both these acids are known to be excitatory neurotransmitters in many sites of the mammalian CNS (Fonnum, 1984; Storm-Mathisen *et al.*, 1983; Watkins and Evans, 1981). Niederlehner *et al.* (1982) reported that the blood level of lidocaine at the onset of the lidocaine-induced convulsion in rats was 19μg/ml (81μM). This is nearly the same level of concentration necessary to begin the process of inhibiting the release of amino acids from synaptosomes in this study.

The result indicates that the IC_{50} of lidocaine for the relase of GABA is significantly lower than the IC_{50} of lidocaine reported by Ikeda *et al.*(1983) This difference may result from using veratrine in the present experiment, while potassium was used by Ikeda *et al.* to induce the release of neurotransmitters. In fact, veratrine has certain advantages over potassium. Since veratrine depolarize the synaptosomal membrane by increasing the influx of Na^+ ion (Narahashi, 1986), more physiological effect can be obtained with veratrine in comparison to potassium which simply reduces the K^+ diffusion potential of the membrane (Cunningham and Neal, 1981). Moreover, veratrine may selectively release GABA from the neurons (Minchin, 1975; Neal and Bowery, 1979).

Propranolol, which can stabilize membranes (Weiner, 1985), also reduced the release of the amino acid neurotransmitters and most effectively blocked the GABA release among the three amino acids. Some anticonvulsants such as phenytoin, carbamazepine and lidocaine have several similar clinical effects and are nearly identically effective as anticonvulsants. They prevent maximal electroshock seizures, but not pentylenetetrazole seizures (Krall *et al.*, 1978). These anticonvulsants as well as lidocaine also block voltage-sensitive sodium channels (Willow *et al.*, 1984). Previously Ferrendelli and Daniels-McQueen (1982) reported that

lidocaine, phenytoin and carbamazepine reduced stimulated Ca^{++} uptake into synaptosmes in a similar manner.

In large doses, carbamazepine and phenytoin, like lidocaine, produce CNS excitations (Rall and Schleifer, 1985). Norepinephrine was reported to block striatal GABA release (Van der Heyden *et al.,* 1981). However, both norepinephrine and 5-HT reduced the release of amino acid neurotransmitters in a qualitatively different fashion when compared to the effect of lidocaine and propranolol in this study. Thus, preferential inhibition of the GABA release might be a common characteristic of lidocaine-like membrane stabilizers. The disappearance of synaptically released GABA (Hamberger *et al.,* 1981; Okamato and Namima, 1978) and possibly glutamic acid and aspartic acid may be due to their reuptake into nerve terminals and glial cells through Na^+-dependent transport mechanism (Levi, 1972; Simon *et al.,* 1974). GABA and glutamic acid carbon skeletons which were again taken up into the nerve terminals were glutamic acid (Reubi, 1980; Shank and Aprison, 1979; Shank and Aprison, 1981; Walaas, 1981), malate or alpha-ketoglutarate (Shank and Campbell, 1984). Lidocaine exerted no significant change in the uptake of all three amino acids in the concentrations used in this study.

Several reports insist that lidocaine can alter the receptor binding of several neurotransmitters (Faihurst *et al.,* 1980) as well as the activity of various membrane-bound enzymes (Hudgins and Bond, 1984; Haque and Poddar, 1983). Lidocaine at concentrations above 1 mM also reduced GABA binding and glutamic acid binding, This concentration, however, is not comparable to the concentration observed at the onset of the lidocaine-induced convulsion. Therefore, this change may not be responsible for this particular effect of lidocaine.

The neurotransmitter release can be prompted by either K^+ or veratrine from synaptosomes (Bradford *et al.,* 1973; Takagaki, 1976) and brain slices (Neal, 1979). In this experiment, lidocaine displayed a strikingly different effect on the K^+-induced amino acid release than the effects of the veratrine-induced release. A much higher concentration of lidocaine was needed to halt the K^+-induced amino acid release than was necessary for the veratrine-induced release. Moreover, the aspartic acid and glutamic acid releases were more sensitive to lidocaine than the GABA release. The veratrine-induced release requires an influx of Na^+ in addition to a mobilization of internally stored Ca^{++}. However, although the K^+-induced release does not require the Na^+ Influx, it does require extracellular Ca^{++} (Cunningham and Neal, 1981; Ferrendelli and Daniels-Mcqueen, 1982). Thus, the blocking of the K^+-induced release by lidocaine might result from decreased Ca^{++} conductance (Ferrendelli and Daniels-Mcqueen, 1982). The absence of extracellular Ca^{++} also enhanced the veratrine-induced release of many amino acid neurotransmitters (Cunningham and Neal, 1981; Benjamin and Quastel, 1972). In contrast to amino acids, the norepinephrine and acetylcholine release by veratrine was reduced in Ca^{++}-free medium (Cunningham and Neal, 1981; Schoffelmeer and Mulder, 1983). These studies indicate that the amino acid release is not so dependent on extrcellular Ca^{++} as norepinephrine and acetylcholine.

In this study, the difference in the requirements of Na^+ and extracellular Ca^{++} may have resulted in the differential inhibition of neurotransmitters. The reduction of Na^+ conductance by lidocaine may have had a greater impact on the GABA release than did other amino acids and norepinephrine. In the case of the K^+-induced release, lowering Ca^{++} conductance through high concentrations of lidocaine may also have had a greater impact on aspartic acid and glutamic acid release than did the GABA relase.

Toxic doses of lidocaine induce convulsions in many laboratory animals. The mode of lidocaine-induced convulsion has been studied, yet the clear mechanism is unknown. The present study shows that the release of inhibitory neurotransmitters such as GABA is more potently inhibited by lidocaine than that of excitatory neurotransmitters from the synaptosomes. Therefore, one could speculate that lidocaine may produce a similar effect in animals CNS and by doing so may induce convusion. One may argue that it would be somewhat unreasonable to extrapolate the *in vitro* data to *in vivo* However, many investigations have indicated that the *in vitro* veratrine-induced release of the neurotransmitters from the synaptosomes is very similar to the physiologic release of neurotransmitters.

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═국문초록═

Lidocaine이 아미노산 신경전도물질의 유리, 수용체 결합, 및 섭취에 미치는 효과에 관한 시험관내 실험에 관한 연구

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Lidocaine 투여에 의한 전신경련의 작용기전을 추구하고자 흰쥐의 전체뇌를 또는 선조체, 해마, 및 중뇌를 부위별로 적출하여 synaptosomes를 마련하고 20μM veratrine 또는 50μM K^+의 첨가에 의한 신경 전달물질(Aspartic acid, Glutamic acid, GABA, Norepinephrine)의 유리촉진작용에 미치는 lidocaine, propranolol, norepinephrine 또는 serotonin의 억제효과를 관찰하였고 [^{3}H]-GABA와 [^{3}H]-glutamic acid의 synaptosomes로의 섭취에 미치는 lidocaine의 영향도 관찰하였다. 아울러 crude synaptic membrane을 이용하여 [^{3}H]-GABA와 [^{3}H]-glutamic acid의 수용체 결합에 미치는 lidocaine의 작용도 실험하여 다음과 같은 결과를 얻었다.

1. Lidocaine과 propranolol은 veratrine에 의한 aspartate, glutamate, GABA 및 norepinephrine의 유리를 억제하였고, 그중 GABA 유리에 대한 억제작용이 가장 현저하였다.

2. Norepinephrine과 serotonin은 100μM의 농도에서 veratrine에 의한 aspartate, glutamate 및 GABA의 유리촉진 작용을 억제하였다.

3. Lidocaine은 veratrine에 의한 아미노산 유리촉진 효과에 대해서 보다 과 K^+에 의한 유리촉진 효과를 더욱 약하게 억제하였고 특히 GABA 유리에 대한 억제작용이 가장 약했다.

4. GABA와 glutamic acid의 수용체 결합과 synaptosomes로의 섭취는 1 mM 이하의 lidocaine 농도에서 크게 변화가 없었다.

이상의 결과로 보아 신경전도물질의 veratrine에 의한 유리가 과 K^+에 의한 유리보다 더욱 생리적이라는 점을 고려한다면, lidocaine 경련은 lidocaine이 흥분성 전도물질인 aspartate나 glutamate보다 억제성 전도물질인 GABA의 유리를 더욱 현저하게 억제함으로서 나타남을 시사한다