Some Correlations between Local Anesthetic-Induced Convulsions and γ -Aminobutyric Acid in Rat Spinal Cord

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ABSTRACT—The effects of local anesthetics (procaine and lidocaine) on the γ -aminobutyric acid (GABA) and L-glutamic acid (Glu) levels in rat spinal cord were studied during the convulsive process. The present study also investigated the influence of central GABA manipulations on the local anesthetic-induced convulsions. An increase in spinal GABA levels was observed at the preconvulsive and convulsive states after administration of procaine (170 mg/kg, i.p.) or lidocaine (120 mg/kg, i.p.), which induced clonic convulsions; in the depressive state, GABA levels returned to normal; in all states, Glu levels were unchanged. Semicarbazide (25–100 mg/kg, i.p.), a glutamic acid decarboxylase inhibitor, produced a decrease in spinal GABA content and strongly enhanced both local anesthetic-induced convulsions as shown by a shortening of the latency and an increase in the mortality. Aminooxyacetic acid (AOAA; 10–40 mg/kg, i.p.), a GABA transaminase inhibitor, dose-dependently increased spinal GABA content and markedly suppressed procaine-induced convulsions. However, lidocaine-induced convulsions were enhanced by AOAA. These results suggest that the spinal GABA neuron may respond to the convulsions induced by local anesthetics. Furthermore, there is a clear relationship between spinal GABA content and procaine-induced, but not lidocaine-induced, convulsions.

Although local anesthetics have anticonvulsant effects in low doses, higher doses of these agents have toxic effects on the central nervous system (CNS), producing restlessness, convulsions, and respiratory failure (1). It is generally agreed that the convulsant effects of local anesthetics are due to a selective depression of inhibitory neurons, allowing enhancement of the effect of excitatory neurons (2, 3).

Several investigators have pursued a correlation between local anesthetic-induced convulsions and changes in inhibitory neurotransmitters (4–9). However, the results obtained are not necessarily the same for all local anes-

thetics. Norepinephrine (NE), dopamine (DA), and serotonin (5-HT) are normally chosen as possible inhibitory neurotransmitters in the CNS. For example, Ciarlone and Juras (7) reported that lidocaine induced both a depletion of DA and 5-HT in some brain areas and an elevation in others, while NE caused only an elevation. Procaine produced a widespread elevation of DA and 5-HT in all brain regions; its effect on NE was largely confined to a few brain structures. Relative to the number of studies on monoamines (10, 11), there have been few investigations on γ -aminobutyric acid (GABA), a major inhibitory neurotransmitter in the CNS, during local anes-

thetic-induced convulsions, and little attention has been paid to the time-course change in GABA levels after administration of local anesthetics.

It is recognized that local anesthetic seizure discharges emanate mainly from the amygdala and hippocampus (12–14). However, the spinal cord is one of the primary active sites in the motor functions and the neurotransmitters in the spinal cord as well as in the brain may be important in the local anesthetic-induced convulsive process. We recently demonstrated that significant elevations of DA and 5-HT levels in the spinal cord occur during the procaine-induced convulsive process (15).

In this study, we attempted to determine whether or not local anesthetics affect GABA and L-glutamic acid (Glu) levels in the spinal cord and to investigate the influence of GABA manipulations on the local anesthetic-induced convulsions.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 180–200 g, were used. They were kept at a constant room temperature (23–25°C) with a 12 hr light-dark cycle (light period: 6:30 A.M.–6:30 P.M.) and maintained on commercial laboratory chow and tap water for at least 5 days before being used.

Chemicals

The following drugs were used: lidocaine HCl (Xyllocaine[®], 10% solution, Fujisawa Pharm. Co., Ltd., Japan); aminooxyacetic acid hemihydrochloride (AOAA), semicarbazide HCl (Wako Pure Chem. Ind. Co., Ltd., Japan); GABA, GABAase, procaine HCl, Glu, NADP, and NAD (Sigma Chemical Co., U.S.A.); and glutamic acid dehydrogenase (Boehringer Mannheim, Germany). All other chemicals used were of analytical reagent grade.

Drug treatment

AOAA, semicarbazide, and procaine were

dissolved in distilled water. These drugs were administered intraperitoneally to rats at their appropriate dosages in a volume of 0.1 ml per 100 g body weight. Lidocaine was used as such and administered in a volume of 0.12 ml per 100 g body weight when given to rat at a dose of 120 mg per kg. The test times of drugs are presented in the tables and figures.

Observation of convulsions

The convulsive behavioral changes were recorded as follows: running motions of the limbs, respiration, convulsion characteristics (clonic/tonic), incidence of convulsions, onset time (latency) and duration time of convulsions, and recovery or death. The experiments were usually performed between 11:30 A.M. and 4:00 P.M. to avoid circadian variations in the endogenous levels of neuroactive transmitters. The experimental observation periods were for at least 1 hr.

Preparation of tissue

After the rats were decapitated at each time point, the spinal cords were quickly removed and frozen in acetone and dry ice. The frozen tissues were weighed and homogenized in 5 ml of cold 75% ethanol with a polytron homogenizer. The homogenate was centrifuged at $15,000 \times g$ for 20 min at 4°C, and the supernatant was evaporated to dryness. The dried sample was resuspended in 2 ml of cold distilled water, and the clear supernatant was obtained for the assay of GABA and Glu by centrifuging as described above.

Measurement of GABA and Glu

The levels of GABA and Glu were measured according to the enzymatic method described by Graham and Aprison (16) with some modifications. For the GABA assay, the reagent solution contained 50 mM sodium pyrophosphate buffer (pH 8.4), 6 mM α -ketoglutarate, 6 mM mercaptoethanol, 0.1 mM NADP, and GABAase (mixture of GABA transaminase and succinic semialdehyde dehydrogenase, 2 units/ml). A 0.5 ml-aliquot of this reagent and 0.1 ml of sample solution

were incubated for 45 min at 38°C. Then 0.1 ml of 0.2 M sodium phosphate solution was added and incubated for 15 min at 60°C. A 0.1-ml aliquot was removed from the incubation mixture and heated together with 0.1 ml of 0.03% H_2O_2 in 10 N NaOH solution for 10 min at 60°C. After adding 3 ml of distilled water, the fluorescence of the NADP product produced from the conversion of NADPH by alkaline-peroxide treatment was measured with a fluorescence spectrophotometer (Hitachi F-200). In the Glu assay, 0.1 ml of sample solution and 1 ml of 0.5 M glycine-hydrazine buffer (pH 8.6) were mixed. To this solution, 0.02 ml of 10 mM NAD and 0.02 ml of glutamic acid dehydrogenase (10 mg/ml) were added, and the mixture was incubated for 30 min at 30°C. The fluorescence of the reaction product NADH was measured. The contents of GABA and Glu were determined by comparison with the standard curves for GABA and Glu.

Statistics

Statistical analysis between experimental groups of data were performed by Dunnett's multiple comparisons.

RESULTS

Relationship between the doses of local anesthetics and convulsions

Procaine administered to rats at doses greater than 130 mg/kg, i.p. produced clonic convulsions following ataxia and the loss of the righting reflex at 5–8 min after the injection. The frequency of convulsions increased in proportion to the dose of procaine (Fig. 1). The mortalities of rats treated with 170 and 190 mg/kg of procaine were 0 and 75%, respectively. A similar convulsive pattern was produced by lidocaine in the range of 80–120 mg/kg (Fig. 1). Many rats injected with 140 mg/kg of lidocaine assumed the opisthotonic position and died during their convulsions. Thus, in the subsequent experiments, procaine and lidocaine were used at doses of 170 and 120 mg/kg, i.p., respectively. From the con-

Fig. 1. Dose-response relationship between local anesthetic dosage and incidence of convulsions in rats. Procaine (●) and lidocaine (○) were administered to rats at the indicated dosages, and the convulsions induced by the two drugs were measured. The incidence of convulsions (ordinate) is shown as the percent of rats that exhibited convulsions per total rats studied. Abscissa: doses (mg/kg, i.p.) of procaine or lidocaine. $n = 5-8$ for each group.

vulsive behavioral changes after administration of procaine or lidocaine, the following classifications of their behavioral state were made: 1) nonconvulsive state (2 min after administration), 2) preconvulsive state (4 min), 3) convulsive state (6 and 8.5 min), and 4) depressive state (30 and 40 min).

GABA and Glu content in rat spinal cord after administration of local anesthetics

Spinal GABA levels were increased in the preconvulsive and convulsive states after administration of procaine (Fig. 2). The maximum GABA level was $1.23 \mu\text{mol/g}$. Increased GABA levels were restored to normal during the depressive state. After administration of lidocaine, significant increases in spinal GABA levels were also produced between the preconvulsive state and the convulsive state (Fig. 2). No significant alterations in Glu content were detected in the spinal cord examined after administration of procaine or lidocaine (Fig. 2).

Fig. 2. Effects of local anesthetics on γ -aminobutyric acid (GABA) and L-glutamic acid (Glu) content in rat spinal cord. After the application of procaine (170 mg/kg, i.p., left panel) and lidocaine (120 mg/kg, i.p., right panel), rats were sacrificed at the times indicated on the abscissa, and spinal GABA and Glu contents were measured. Each column represents the mean $\pm$ S.D. of 5 to 6 rats. * $P < 0.05$, ** $P < 0.01$, compared with the control values.

Effects of semicarbazide and AOAA on local anesthetic-induced convulsions and spinal GABA content

Semicarbazide intensified the convulsions induced by both procaine and lidocaine, as shown by a shortening of the latency and an increase in the mortality (Table 1). The spinal GABA contents of rats injected with 25 and 50 mg/kg of semicarbazide were not different from the controls, but 100 mg/kg of semicarbazide decreased spinal GABA content by 19% (Table 2). AOAA dose-dependently re-

duced the incidence of procaine-induced convulsions and prolonged the latency (Table 1). Lidocaine-induced convulsions after administration of AOAA were very different from procaine-induced ones (Table 1). The prolongation of the latency was much briefer than that in the procaine group. Pretreatment with AOAA did not reduce the incidence of lidocaine-induced convulsions; in fact, the mortality was markedly increased. AOAA also prolonged the duration of both convulsions, more with lidocaine than with procaine.

Table 1. Effects of semicarbazide and aminooxyacetic acid (AOAA) on procaine- and lidocaine-induced convulsions in rats

	Drug	Dose (mg/kg, i.p.)	Frequency of convulsions		Latency of onset (min)	Duration of convulsions ^b (min)	Mortality		
			N ^a	%			N ^a	%	
Procaine	Control	—	10/10	100	5.08 ± 1.05	16.6 ± 1.82	0/10	0	
	Semicarbazide	25	5/ 5	100	3.85 ± 0.47	16.1 ± 1.34	0/ 5	0	
		50	5/ 5	100	3.48 ± 0.86*	19.8 ± 1.06* (2.89 ± 1.05) ^c	3/ 5	60	
		100	5/ 5	100	2.18 ± 0.82**	19.3 (2.52 ± 0.43)	4/ 5	80	
	AOAA	10	5/ 5	100	6.47 ± 1.77	19.2 ± 1.37*	0/ 5	0	
		20	6/10	60	8.18 ± 2.00*	16.1 ± 2.32	0/10	0	
		40	3/10	30	11.20 ± 2.05**	20.1 ± 1.72**	0/10	0	
	Lidocaine	Control	—	10/10	100	4.08 ± 0.46	42.5 ± 3.44	0/10	0
		Semicarbazide	25	5/ 5	100	3.67 ± 0.86	43.7 ± 1.82 (1.29 ± 0.30)	2/ 5	40
50			5/ 5	100	3.30 ± 1.19	41.3 ± 2.89 (1.81 ± 0.75)	3/ 5	60	
100			5/ 5	100	1.87 ± 0.36**	(2.20 ± 1.29)	5/ 5	100	
AOAA		10	5/ 5	100	5.32 ± 1.47	39.6 ± 8.67	1/ 5	20	
		20	10/10	100	6.05 ± 1.97**	51.2 ± 6.13 (1.83 ± 0.47)	5/10	50	
		40	10/10	100	6.43 ± 1.27**	58.6 ± 11.7** (1.35 ± 0.34)	6/10	60	

After pretreatment with AOAA and semicarbazide, procaine (170 mg/kg, i.p.) and lidocaine (120 mg/kg, i.p.) were injected into rats, and the convulsive parameters induced by the two drugs were measured. AOAA and semicarbazide were administered 1 and 2 hr before being challenged with procaine and lidocaine. Each value represents the mean ± S.D. *P < 0.05, **P < 0.01, compared with the control values. ^aNumber of rats that exhibited convulsions or death/number of rats studied. ^bThe duration times were measured from the occurrence of the convulsions to the recovery of the righting reflex. ^cThe values in parentheses show the time to death after convulsions.

Table 2. Effects of semicarbazide and aminooxyacetic acid (AOAA) on γ -aminobutyric acid (GABA) content in rat spinal cord

Drug	Dose (mg/kg, i.p.)	No. of rats	GABA content (μ mol/g tissue)
Control	—	5	1.08 ± 0.09
Semicarbazide	25	6	1.02 ± 0.08
	50	6	0.95 ± 0.09
	100	6	0.88 ± 0.08*
Control	—	5	1.05 ± 0.09
AOAA	10	6	1.31 ± 0.14
	20	6	1.80 ± 0.15**
	40	6	2.29 ± 0.25**

Rats were treated with AOAA or semicarbazide and sacrificed at 1 or 2 hr later. Then the spinal cord was removed, and the GABA content was measured. Each value represents the mean ± S.D. *P < 0.05, **P < 0.01, compared with the control values.

The spinal GABA levels after administration of AOAA were significantly increased in proportion to the AOAA dosage (Table 2).

An increase in spinal GABA levels induced by the two local anesthetics was suppressed by pretreatment with semicarbazide, and the spinal GABA levels increased by a single administration of AOAA were unchanged after an additional administration of the two local anesthetics (Table 3).

Spinal GABA content and local anesthetic-induced convulsions

On the basis of the data from the convulsions induced by the two local anesthetics and the spinal GABA content after administration of semicarbazide or AOAA, we graphed the correlation between the changes in spinal GABA content and the local anesthetic-induced convulsions. The latency of procaine-induced convulsions shortened with a decrease in spinal GABA content of the procaine-injected rats and prolonged with a progressive increase in the GABA content; a linear correlation was found between spinal GABA content and the latency (Fig. 3). The inci-

dence of convulsions was identical (100%) after administration of procaine at spinal GABA levels below about $1.3 \mu\text{mol/g}$; above this point it gradually decreased with an increase in the GABA content. On the other hand, the correlation between lidocaine-induced convulsions and spinal GABA content was biphasic (Fig. 3). The pattern of latency of lidocaine-induced convulsions showed a long prolongation as the GABA levels began to increase, but the prolongation shortened and there was no protection against convulsion when the spinal GABA content reached more than $1.3 \mu\text{mol/g}$.

DISCUSSION

The first finding of this study is that the convulsions induced by the two local anesthetics studied are accompanied by significant increases in spinal GABA levels, although the levels of Glu, a metabolic precursor of GABA and an excitatory neurotransmitter in the CNS, are unchanged during the convulsive process. This result indicates that the GABAergic neuron is involved in the local

Table 3. Effects of the combination of semicarbazide, aminooxyacetic acid (AOAA) and local anesthetics on γ -aminobutyric acid (GABA) content in rat spinal cord

Drug (mg/kg, i.p.)	No. of rats	GABA content ($\mu\text{mol/g}$ tissue)
Control	4	1.05 ± 0.08
Semicarbazide (50) ^a	6	0.95 ± 0.09
Semicarbazide (50) + Procaine (170)	4	1.02 ± 0.11
Semicarbazide (50) + Lidocaine (120)	4	0.92 ± 0.08
Control	4	1.06 ± 0.09
AOAA (20) ^a	6	$1.80 \pm 0.15^{**}$
AOAA (20) + Procaine (170)	4	$1.74 \pm 0.10^{**}$
AOAA (20) + Lidocaine (120)	4	$1.66 \pm 0.15^{**}$

After pretreatment with AOAA and semicarbazide, the rats were treated with procaine and lidocaine and sacrificed 6 min later. Then the spinal cord was removed, and the GABA content was measured. AOAA and semicarbazide were administered 1 and 2 hr before being challenged with the two local anesthetics. Each value represents the mean $\pm$ S.D. ^aThe GABA content after a single administration of semicarbazide or AOAA is listed from Table 2. ^{**} $P < 0.01$, compared with the control values.

Fig. 3. Correlation between spinal γ -aminobutyric acid (GABA) content and local anesthetic-induced convulsions. Rats were divided into two groups. In one group, rats were pretreated with aminooxyacetic acid (AOAA) (10–40 mg/kg, i.p.) or semicarbazide (25–100 mg/kg, i.p.) and then procaine (170 mg/kg, i.p., upper panel) and lidocaine (120 mg/kg, i.p., lower panel) were injected 1 or 2 hr later. After administration of the two local anesthetics, the convulsive parameters induced by the two drugs were measured. In another group, rats were sacrificed at 1 or 2 hr after administration of AOAA or semicarbazide, and spinal GABA content was measured. The GABA content after administration of AOAA or semicarbazide is the average of 6 rats per group. The latency (○) and the incidence (●) of convulsions is the average of 5 to 10 rats per group; these values are partially listed in Tables 1 and 2. The arrow shows the control level. The control values were as follows (mean $\pm$ S.D.): GABA content, 1.08 ± 0.09 μ mol/g; latency after procaine administration, 5.08 ± 1.05 min; latency after lidocaine administration, 4.08 ± 0.46 min.

anesthetic-induced convulsions, but that the excitatory Glu neuron is not. The second finding is that the convulsions induced by procaine can be inhibited by AOAA which produced an increase in spinal GABA levels and potentiated by semicarbazide which produced a decrease in spinal GABA levels, while the convulsions induced by lidocaine are different with respect to their sensitivity to pharmacological manipulations of the GABA levels in the spinal cord. Our findings suggest that the convulsions induced by procaine are affected by the variation of central GABA levels in the procaine-injected rats, but that the convulsions induced by lidocaine are not. In spite of the prolongation of the latency, the duration of both convulsions was enhanced by AOAA. These discrepancies may be attributable to the sedative properties of AOAA (17) or to the differing pharmacological effects in the CNS; lidocaine produces sedation while procaine does not (2). Both latency and duration, indicated to be parameters defining the convulsion, are considered to be affected by the activation of the central GABA system. Accordingly, it can be inferred that the level of GABA closely correlates with the latency of the convulsions rather than the duration, since the latency of both convulsions was hyperresponsive to the spinal GABA levels elicited by semicarbazide and AOAA.

There is a quantitative relationship between central GABA levels and seizure susceptibility in some, but not all experimentally produced seizures. γ -Acetylenic GABA and γ -vinyl GABA, which are irreversible GABA transaminase inhibitors, protect mice against seizures induced by audiogenic stimuli, electroshock, isoniazid and strychnine; the degree of seizure protection appears to correlate with elevations in whole brain GABA levels (18, 19), while there is considerable evidence that the increase in central GABA levels and the change in seizure susceptibility are not necessarily parallel (20, 21). For example, there was a remarkable decrease in electroshock seizure incidence in mice during the initially increased GABA levels, but there was no suppression of

such seizures when the GABA content was maximally raised (20). There are several ways to view this problem: (a) the change in GABA may not be directly involved in the anticonvulsant activity; (b) the metabolites of GABA are effective against the convulsions; or (c) the initially increased GABA produces an increase in excitatory factors such as Glu and acetylcholine. However, the metabolites of GABA, γ -hydroxybutyrate and γ -butyrolactone, have no protective effect against the convulsions induced by local anesthetics (10), and the Glu content does not increase after administration of AOAA (20). The hypothesis that GABA is not a nonspecific anticonvulsant is demonstrated by its lack of activity against the convulsions induced by nicotine, strychnine, pentylenetetrazole, and picrotoxin (10). At the present time, it is not clear why the change in lidocaine-induced convulsions after administration of AOAA is different from those induced by procaine.

Recently, we have found that the procaine convulsions are accompanied by significant increases in spinal DA and 5-HT levels; the convulsions are strongly suppressed by the 5-HT precursor 5-hydroxytryptophan and enhanced by the DA precursor 3,4-dihydroxyphenylalanine (15). These previous findings and the results obtained in this study suggest that the variations in spinal GABA content are not a causal factor for the convulsions induced by local anesthetics, but rather an endogenous anticonvulsant factor. This GABA function is supported by the observations that the spinal GABA levels elevated after administration of procaine or lidocaine returned to normal during the depressive state, and that the spinal GABA levels increased by AOAA were no longer changed after an additional administration of the two local anesthetics. Further support for our suggestion is the observation that the amplitude of the rise of spinal GABA content elicited by procaine or lidocaine alone was smaller than the GABA increments due to the administration of AOAA and the observation that the convulsions induced by procaine were strongly suppressed at spinal

GABA levels above about $1.3 \mu\text{mol/g}$, but those induced by lidocaine were not.

It is well-known that the GABAergic neuron and the dopaminergic neuron in the CNS regulate each other (22, 23). However, whether these neurons act on the convulsions induced by the local anesthetics through direct or indirect mechanisms has remained unclear. Further experiments should be made to elucidate these problems involved in the local anesthetic-induced convulsions.

In conclusion, both procaine and lidocaine produce significant elevations of spinal GABA levels in the convulsive process and the variations in GABA levels are an endogenous anticonvulsant factor; however, there is a clear relationship between spinal GABA content and procaine-induced convulsions, which is not found with lidocaine-induced convulsions. Furthermore, the central GABA system is associated with the latency of the convulsions.

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