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Protection from Local Anesthetic-induced Convulsions by γ-aminobutyric Acid

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The effects of gamma-aminobutyric acid (GABA) on the induction of convulsions by local anesthetics were investigated in mice and rats. Intraventricular administration of 0.8–1.6 mg GABA protected rats against convulsions induced by procaine, lidocaine, cocaine, and tetracaine in a dose-related manner. Intraperitoneal γ-acetylenic GABA was also effective against procaine-induced convulsions in mice, but the metabolites of GABA, γ-hydroxybutyrate, and γ-butyrolactone were without effect. Intraventricular GABA, 1.6 mg, delayed the onset of convulsions induced by hydrazine, but had no influence on the incidence of convulsions induced by nicotine, pentylenetetrazol, picrotoxin, or strychnine. These results suggest that the GABA system may be involved in the mechanisms of local anesthetic-induced convulsions. (Key words: Anesthetics, local: cocaine; lidocaine; procaine; tetracaine. Brain: convulsions; gamma-aminobutyric acid.)

A MAJOR COMPLICATION of local anesthetic administration is the development of generalized tonic-clonic convulsion. Electrophysiologic studies in experimental animals have revealed that local anesthetics produce excitatory effects on the limbic system which are most striking in the amygdaloid nuclear complex.[1–3] Furthermore, a recent study by an autoradiographic analysis of cerebral metabolism during lidocaine-induced seizure activity has demonstrated that the hippocampus developed a striking increase in metabolic activity coupled with increased neurophysiologic seizure activity.[4] It is generally agreed that convulsions produced by local anesthetics are not due to direct stimulation, but to selective depression of inhibitory neurones.[5,6] Thus, release from inhibition presumably gives rise to enhanced or unrestrained excitability. This interpretation was supported by an electrophysiologic study which demonstrated that lidocaine blocked inhibitory synapses of rabbit cortical neurones, but had comparatively little effect on excitatory synapses.[7] Lidocaine also facilitates the spinal monosynaptic reflex, suggesting suppression of certain inhibitory spinal functions in preference to excitatory functions.[8]

Based on these concepts, we hypothesize from the biochemical point of view, that local anesthetics block the function of inhibitory neurones by deranging the dynamic metabolism of the neurotransmitter or the interaction with its receptor and the subsequent events. Assuming that γ-aminobutyric acid (GABA) is a transmitter at inhibitory synapses and that benzodiazepines which have potentiating effects on the released GABA action is effective in blocking or treating local anesthetic-induced convulsions,§[9] we designed a study to investigate whether exogenously administered GABA could antagonize local anesthetic-induced convulsions.

Methods

Male dd strain mice weighing 25 to 30 g, and male Wistar rats weighing 140 to 220 g were housed in an animal breeding room where the temperature was maintained at 22 ± 2°C. They had free access to food and water at all times until two hours before experiments. All experiments were carried out between 1 P.M. –4 P.M.

Various drugs as described below were dissolved into 0.9 per cent saline and injected subcutaneously (sc) or intraperitoneally (ip) in a volume of 0.05 ml/10 g to mice and 0.1 ml/100 g to rats. Some were administered into a lateral ventricle according to the procedure of Krammer *et al.*[10] In brief, rats were given intravenous succinylcholine chloride (0.3 mg/kg) and fixed in a stereotaxic device. The animals recovered normal muscle function after about 1 min. A hole in the coronal suture, 1.5 mm lateral to the bregma, was made by an electric motor handpiece and a 27-gauge needle was inserted to a depth of 4 mm from the surface of the skull. Needle placement was verified at the end of the study by dissection after injection of indian ink. Twenty-five microliter of drug solution (*p*H was adjusted to 7.4) was injected. Control animals underwent the same operation and were given an equal volume of 0.9 per cent saline. The operation and injection were completed within a minute after succinylcholine injection. Convulsive responses induced by the injected drugs were evaluated by observing for signs of clonic convulsion.

Procaine, 250 mg/kg (180 mg/kg for mice), lidocaine, 100 mg/kg, cocaine, 55 mg/kg, and tetracaine, 35 mg/kg, were administered intraperitoneally in rats. Other agents used for inducing convulsions in rats were 3 mg/kg nicotine, ip, 60 mg/kg pentylenetetrazol, ip, 5 mg/kg

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§ Braestrup C, Nielsen M: Searching for endogenous benzodiazepine receptor ligands. Trends in Pharmacological Sciences 1:424–427, 1980.

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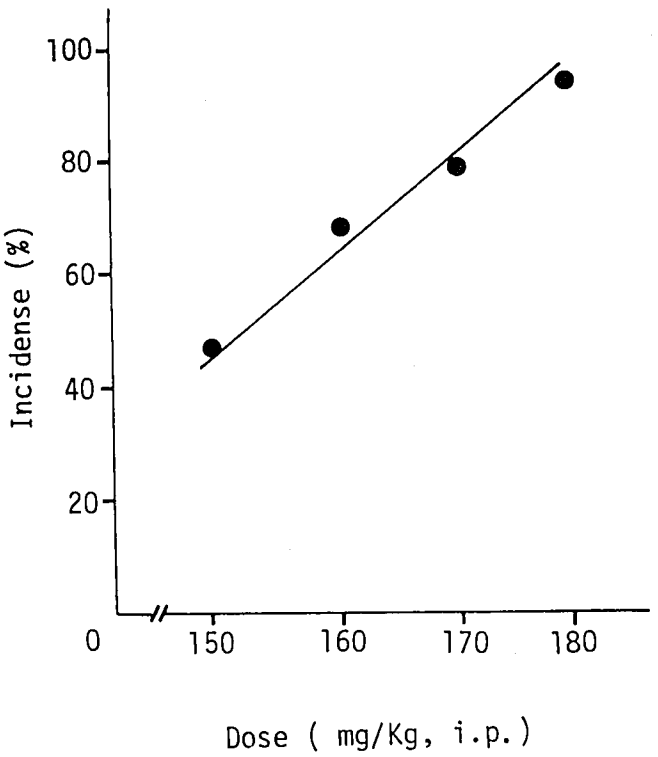

FIG. 1. Dose-response relationship between incidence of convulsions and procaine dosage in mice.

picrotoxin, sc, 1.8 mg/kg strychnine, ip, and 400 mg/kg hydrazine, ip. Used also were GABA, γ-hydroxybutyrate (GHB), γ-butyrolactone (GBL), and γ-acetylenic GABA (GAG) delivered intraperitoneally (mg/kg) or intraventricularly (mg/animal) 15 to 30 min or 4 h before the injection of the convulsive agents in doses indicated in the text. More than 10 rats and 16 mice were used for each group. Statistical analysis was performed by χ^2 analysis of 2×2 contingency table or Student's t test (NS = not significant, $P > 0.05$).

Results

Procaine in doses greater than 150 mg/kg administered intraperitoneally to mice produced ataxia, jumping, clonic convulsion (in some cases, tonic convulsion) at about 5 min after the injection. Frequency of convulsions increased in proportion to the dose of procaine in the range of 150–180 mg/kg as shown in figure 1. The mortality rate at a dose of 180 mg/kg was 30 per cent. A similar convulsive pattern was induced by various local anesthetics in rats. The order of potency of local anesthetics for causing convulsions was in agreement with that for their intrinsic local anesthetic activity. For instance, doses of procaine, lidocaine, cocaine, and tetracaine required to produce more than an 80 per cent incidence of convulsions in rats were 250, 100, 55, and 35 mg/kg, respectively.

Intraperitoneal GABA did not influence the incidence of procaine convulsions in mice, except when administered in extremely large doses (table 1), but when administered intraventricularly 30 min prior to the injection of procaine, the incidence was reduced in a dose related manner. It was similarly effective against lidocaine-, cocaine-, and tetracaine-induced convulsions. Transient depression of spontaneous movement was induced by GABA injection in a dose of 0.8 mg. At higher doses, righting reflex was lost shortly after injection, but re-

TABLE 1. Effect of GABA on Various Local Anesthetic-induced Convulsions

Local Anesthetics (mg/kg)*	Animal Species Studied	GABA Dosage (mg/animal)†	Number of Injection	Number Animals Convulsed	Per Cent Animals Convulsed	Significance‡
Procaine 180 mg/kg	Mouse	0	36	34	94	
		250	16	13	81	NS
		500	16	11	69	<0.05
		1,000	36	19	53	<0.001
		2,000	36	20	56	<0.01
Procaine 250 mg/kg	Rat	0	28	24	86	
		0.8	10	4	40	<0.05
		1.0	10	2	20	<0.001
		1.6	10	0	0	<0.001
Lidocaine 100 mg/kg	Rat	0	10	8	80	
		1.6	10	0	0	<0.01
Cocaine 55 mg/kg	Rat	0	10	8	80	
		1.6	10	0	0	<0.01
Tetracaine 35 mg/kg	Rat	0	10	10	100	
		1.6	10	1	10	<0.001

* Drugs were administered intraperitoneally.

† Drugs were administered intraventricularly except dosage in mice represents mg/kg intraperitoneally. GABA was administered 30 minutes prior to local anesthetic administration.

‡ Determined by χ^2 analysis of 2×2 contingency table.

TABLE 2. Effect of γ-Hydroxybutyrate (GHB), γ-Butyrolactone (GBL), and γ-Acetylenic GABA (GAG) on Procaine-induced Convulsions

Procaine	Animal	Control	GHB		GBL	GAG
			400 mg/kg ip	1.9 mg/rat ivc	400 mg/kg ip	100 mg/kg ip
250 mg/kg	Rat	80 100	90 (NS)	80 (NS)	80 (NS)	
180 mg/kg	Mouse	90				30 (<0.001)

Procaine was administered at 15 min after GHB and GBL injection, and 4 h after GAG injection.

Results were expressed as per cent incidence of convulsing. Ten rats and 20 mice were used for each group. Statistical analysis was performed as shown in table 1.

turned in 3 min, while slight depression of spontaneous motor activity was observed at 30 min after administration. Both γ-hydroxybutyrate and γ-butyrolactone, metabolites of GABA, caused depression of spontaneous motor activity or loss of righting reflex for a period of 30 min. However, neither influenced the incidence of convulsions (table 2). The effect of γ-acetylenic GABA was examined because it increases brain GABA concentration by irreversible inhibition of GABA transaminase (GABA-T) activity.[11] γ-Acetylenic GABA protected mice from convulsions induced by procaine (table 2).

Intraventricularly administered GABA had no effect on the incidences of convulsions induced by nicotine, pentylenetetrazol, picrotoxin, or strychnine (table 3), and did not alter the incidence of convulsions induced by hydrazine, but did delay its onset.

Discussion

GABA is considered to be a major inhibitory transmitter in the brain and plays an important role in seizure etiology. Hydrazine derivatives, certain pyridoxine antagonists, allylglycine, and 3-mercaptopropionic acid are inhibitors of glutamic acid decarboxylase (GAD), an enzyme necessary for GABA synthesis, and are potent convulsants. Other convulsive agents, *e.g.*, bicuculline and picrotoxin, are thought to act as specific inhibitors of postsynaptic GABA receptors and GABA ionophores, respectively.[12,13] Conversely, some anticonvulsants, *e.g.*, aminooxyacetic acid, n-depropylacetate, γ-acetylenic GABA, and γ-vinyl GABA share the inhibitory activity of GABA-T and increase brain GABA concentration.[14,15] Other investigators have suggested the importance of GAD activity for controlling GABA concentration in the synaptic cleft and for seizure protection.[16,17] This evidence suggests that modulation of the GABA system may be closely associated with the development of or protection from seizure, although the majority of convulsants and anticonvulsants exert their activity without involving the GABA system.

In the present studies, we demonstrated that the induction of convulsions by various local anesthetics was prevented by intraventricular GABA administration in rats. γ-Hydroxybutyrate and γ-butyrolactone are GABA metabolites and are known to have anesthetic properties accompanied by a striking increase in brain dopamine levels.[18] These metabolites caused loss of righting reflex in the rats in this experiment. Nevertheless, procaine still produced convulsions in rats pretreated with anesthetic doses of these metabolites. These results suggest that the anticonvulsant activity of GABA is not due to the action of these metabolites, nor is it secondary to their sedative or paralytic effect. That GABA is not a nonspecific anticonvulsant against all experimentally produced convulsions is demonstrated by its lack of activity against convulsions induced by nicotine, pentylenetetrazol, and

TABLE 3. Effect of Intraventricular Injection of GABA on Convulsions Induced by Various Types of Convulsants

	Dose and Route (mg/kg)	Per Cent Incidence of Convulsion	
		Control (n = 10)	GABA 1.6 mg/rat ivc (n = 10)
Nicotine	3 ip	80	90
Pentylenetetrazol	60 ip	80	70
Picrotoxin	5 sc	100	100
Strychnine	1.8 ip	70	100
Hydrazine	400 ip	90	80
		(19.3 ± 1.7*)	(61.0 ± 6.2*†)

* Time in min between injection of hydrazine and appearance of convulsion (Mean ± SEM).

† Significant difference from control by Student *t* test, $P < 0.01$.

strychnine. Although, both hydrazine and picrotoxin are convulsive agents which involve the GABA system, GABA was only effective against convulsions induced by hydrazine. The difference in anticonvulsant activity may be explained by the different sites of action of the two convulsants. Namely, hydrazine blocks the GABA system by decreasing GABA concentration in the brain; this can be restored by exogenously administered GABA. On the other hand, picrotoxin acts on the postsynaptic GABA ionophore, thereby preventing augmented GABA from exerting its inhibitory effects. Our results are in agreement with the reported lack of effect of γ-acetylenic GABA on picrotoxin-induced seizures.[11] γ-Acetylenic GABA and γ-vinyl GABA are catalytic irreversible inhibitors of GABA-T.¶ Schechter *et al.*[14] reported that the increase in brain GABA levels induced by these inhibitors correlated well with their antiseizure activity against audiogenic seizure. We also demonstrated through our study that local anesthetic-induced convulsions were prevented by γ-acetylenic GABA. This evidence strongly supports the hypothesis that local anesthetics may derange the brain GABA function and disrupt inhibitory input, resulting in increased exitation and convulsions.

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