Changes in Convulsion Susceptibility of Lidocaine by Alteration of Brain Catecholaminergic Functions

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ABSTRACT—Influences of the manipulation of brain catecholaminergic neuronal activity on the incidence of lidocaine-induced convulsions in mice were studied and compared with those of pentylenetetrazol (PTZ)-induced convulsions. α-Methyl-p-tyrosine (α-MPT) decreased both brain noradrenaline (NA) and dopamine (DA) levels, and disulfiram decreased the NA level and increased the DA level. The incidence of lidocaine-induced convulsions was decreased by treatments with α-MPT and disulfiram, while that of PTZ was increased by either treatment. The incidence of lidocaine-induced convulsions was slightly, but not significantly increased by L-dihydroxyphenylalanine (L-DOPA), although the brain DA level was increased by L-DOPA. Methamphetamine and desipramine increased the incidences of lidocaine-induced convulsions. These results may suggest that brain catecholaminergic neurons, differing from their role in inhibiting control of PTZ-seizure, act to facilitate lidocaine-induced convulsions.

A major pharmacological sign of toxicity from local anesthetic administration is an excitation of the central nervous system (CNS) as in the development of general tonic-clonic convulsions, although local anesthetics have an anticonvulsant effect in low doses (1). Intravenously infused lidocaine has been shown to cause a tetraphasic action on CNS electrical activity (initially a decrease; secondarily, an increase and then decrease; and finally, an increase in reticular neuronal firing) in cats (2). Stimulation of the CNS by local anesthetics is characterized by the activation of limbic discharges which is most striking in the amygdaloid nuclear complex (3–6) and the increase in metabolic activity which is notable in the hippocampus (7). It is generally agreed that local anesthetics induce convulsions by depressing inhibitory neurons, thereby permitting facilitation of excitatory neurons. This interpretation is suggested by demonstrations that lidocaine blocked inhibitory synapses in rabbit cortical neurons, but had comparatively little effect on excitatory synapses (8), and that lidocaine facilitated the spinal monosynaptic reflex, suggesting suppression of certain inhibitory spinal functions in preference to excitatory functions (9). γ-Aminobutyric acid (GABA) is considered to be a major inhibitory neurotransmitter in the mammalian CNS. We have demonstrated that local anesthetics inhibited GABA release from synaptosomes (10) and that intraventricularly administered...
GABA could protect rats against convulsions induced by local anesthetics (11). This evidence suggests that the action of local anesthetics on the brain GABA system may be involved in the development of local anesthetic-induced convulsions.

It is also suggested that brain monoamines serve as inhibitory neurotransmitters in convulsions induced by electroconvulsive shock and by certain drugs. The seizures induced by electroconvulsive shock were potentiated in animals depleted of brain monoamines (12, 13). Mason and Corcoran examined the influence of regional CNS depletion of noradrenaline (NA), without affecting dopamine (DA) and serotonin (5-HT) levels, by injecting 6-hydroxydopamine into discrete areas of the brain (14–17). Their results demonstrated that depletion of NA in the descending fibers innervating the spinal cord increased the incidence of electroconvulsive shock, but not pentylentetrazol (PTZ) seizures, and depletion of NA in the ascending forebrain fibers increased susceptibility to PTZ seizures. Recently, many studies support the thesis that seizure susceptibility to PTZ or electroshock is increased by treatments known to deplete brain NA levels and may be decreased by elevating the level of NA. 5-HT appears to have similar action with that of NA, but DA has little effect. On the other hand, there were few studies on the relationship between local anesthetic-induced convulsions and brain amines. It has been suggested that endogenous brain stores of 5-HT may play role in the CNS toxicity of local anesthetics because an increase in brain content of 5-HT intensified local anesthetic-induced convulsions, while a decrease in 5-HT content increased the threshold (18, 19). Ciarlone reported that DA depletion lowered lidocaine and procaine-induced seizure thresholds (20), while concurrent depletion of brain NA and DA had no effect on lidocaine seizure threshold (21). Therefore, to ascertain the implication of aminergic control of local anesthetic-induced convulsions, the influence of treatments which could activate or depress the function of brain monoamine neurons on the incidence of local anesthetic convulsions was examined. The results were compared with those of PTZ seizures because the action of PTZ on the GABA system, in addition to the direct effect on membrane properties related to excitability, is thought to be included in PTZ's seizure mechanism (22).

MATERIALS AND METHODS

Male ddY-strain mice weighing from 20 to 30 g were used. The experiments were performed in the afternoon because of the effects of circadian rhythms on these experiments. They had free access to food and water at all times prior to a point three hours before the experiments. Drugs were administered intraperitoneally at doses of 0.05 ml/10 g body weight. Disulfiram was suspended in 3% Tween 80, and L-dihydroxyphenylalanine (LDOPA) was suspended in 0.3% carboxymethyl cellulose solution. Other drugs were dissolved in saline. In control populations or in control injections when drugs were not administered, equivalent volumes of vehicle were injected. The dose and the schedule for the treatment of each drug to manipulate the brain catecholamine levels will be described in the text. Doses of methamphetamine (5 mg/kg) and desipramine (20 mg/kg) that increased the motor activity were used. Under these conditions, the effects of drugs on the incidence of drug-induced convulsions were examined. Groups of 10 to 30 mice were used for each treatment, and incidences of clonic convulsions for lidocaine and clonic-tonic convulsions for PTZ in each group were recorded. The statistical analysis of significance of the difference in the incidence of convulsions was made using the chi square test.

For the determination of brain catecholamines, at the time corresponding to the administration of convulsants after various treatments, the brain was isolated and homogenized in ice-cold 0.4 N perchloric acid. Catecholamines were purified by absorption and elution on aluminium oxide, and the NA and DA were assayed fluorometrically by the
method described by Anton and Sayre (23, 24). Values are expressed as the mean ± S.E.M. Statistical analysis of the significance of the difference was performed by Student's t-test.

RESULTS

Lidocaine in doses greater than 60 mg/kg administered intraperitoneally to mice produced ataxia, short loss of the righting reflex and clonic convulsions about 5 to 8 min after the injection. PTZ greater than 50 mg/kg produced facilitation of spontaneous movement, ataxia and clonic and tonic convulsions about 1 to 2 min after the injection. The duration of lidocaine-induced convulsions was shorter than that of PTZ. The CD$_{50}$ of lidocaine was 70 mg/kg and that for PTZ was 55 mg/kg.

Effects of suppression of brain catecholaminergic neurons

$\alpha$-Methyl-p-tyrosine ($\alpha$-MPT), well-known as a tyrosine hydroxylase inhibitor, is commonly used to decrease brain NA and DA content. Six hours after the treatment with $\alpha$-MPT (200 mg/kg), brain NA and DA concentrations were reduced to 55% and 14% of the control levels, respectively (brain NA levels were $0.40 \pm 0.05$ in the control and $0.22 \pm 0.04^*$ in the $\alpha$-MPT-treated mice and brain DA levels were $1.66 \pm 0.37$ in the control and $0.24 \pm 0.06^*$ in the $\alpha$-MPT-treated mice, in $\mu$g/g tissue; $^*P < 0.05$, $n = 10$). In $\alpha$-MPT-treated mice, the incidences of lidocaine-induced convulsions were reduced, and those of PTZ were increased (Fig. 1). The results may suggest that the depletion of brain NA and/or DA produces the opposite effects on lidocaine- and PTZ-induced convulsions. Thus the effects of specific depletion of brain NA on the incidence of two convulsants were examined. Disulfiram, an inhibitor of dopamine-$\beta$-hydroxylase was used for this purpose. When disulfiram was injected, brain NA concentration significantly decreased and DA concentration significantly increased 1 hr after the treatment (brain NA levels were $0.41 \pm 0.04$ in the control and $0.29 \pm 0.01^*$ in the disulfiram-treated mice and brain DA levels were $1.08 \pm 0.03$ in the control and $1.61 \pm 0.09^{**}$ in the disulfiram-treated mice, in $\mu$g/g tissue; $^{*P < 0.05}$, $^{**P < 0.01}$, $n = 10$). After injection with disulfiram, the incidence of lidocaine-induced convolution was reduced (Fig. 2). Conversely, the incidence of PTZ-induced convolution was increased by the treatment. The dose-response relationship of disulfiram on lidocaine- and PTZ-induced convulsions further confirmed the contrasting influence on the effects of the two convulsants; disulfiram attenuated lidocaine convulsions, while it potentiated PTZ convulsions (Fig. 3).

Effects of activation of brain catecholaminergic neurons

Brain DA level increased about 3.5 times that of the control at 30 min after injecting 200 mg/kg of L-DOPA (brain NA levels were $0.42 \pm 0.02$ in the control and $0.51 \pm 0.06$ in the L-DOPA-treated mice, and brain DA levels were $0.90 \pm 0.14$ in the control and 3.33
Fig. 2. Effects of disulfiram on lidocaine- and PTZ-induced convulsions. Disulfiram (200 mg/kg) was injected 1 hr before the administration of the convulsants. N = 10. □ control, ☒ disulfiram. (1) P < 0.05.

Fig. 3. Dose-dependent effects of disulfiram on lidocaine- and PTZ-induced convulsions. Disulfiram (100 mg/kg and 200 mg/kg) was injected 1 hr before the administration of lidocaine or PTZ. N = 20.

Fig. 4. Effects of L-dihydroxyphenylalanine (L-DOPA) on lidocaine- and PTZ-induced convulsions. L-DOPA (200 mg/kg) was injected 30 min before administration of the convulsants. N = 10. □ control, ☒ L-DOPA.

Fig. 5. Effects of methamphetamine (A) and desipramine (B) on lidocaine-convulsions. Methamphetamine (5 mg/kg) was injected 30 min before and desipramine (20 mg/kg) 60 min before the administration of lidocaine. N = 30. □ control, ☒ methamphetamine or desipramine. (1) P < 0.05, (2) P < 0.01, vs. control.
± 0.94* in the L-DOPA-treated mice, in μg/g tissue; *P < 0.05, n = 10). L-DOPA treatment caused a small, but not statistically significant increase in the incidence of both lidocaine- and PTZ-induced convulsions (Fig. 4). The incidence of lidocaine-induced convulsions was increased by methamphetamine which enhances release and inhibits reuptake of NA and DA in the CNS. The tricyclic antidepressant desipramine is a potent inhibitor of NA uptake, but has only a minimal effect on DA uptake. After administration of desipramine, the incidence of lidocaine-induced convolution was raised (Fig. 5).

DISCUSSION

Diminishing the influence of NA, DA or 5-HT in the brain by depleting their content or by blocking the receptors enhances the seizure activity produced by electroshock or PTZ, and increasing their influence may suppress seizures. Thus brain monoamines have inhibitory roles in the convulsions in these seizure models (25).

In the present study, the incidence of lidocaine-induced convulsions in mice decreased when the brain contents of NA and DA were lowered by the administration of α-MPT, an inhibitor of tyrosine hydroxylase, while the incidence of PTZ-induced convulsions increased by this treatment. The increase in the incidence of PTZ-induced convulsions effected by α-MPT agrees with the findings that PTZ-induced convulsions were potentiated by a depletion of brain amines effected by α-MPT or reserpine (14, 26). Thus, the decrease in NA or DA can influence lidocaine- and PTZ-induced convulsions in opposite directions. The converse control by brain amines of lidocaine- and PTZ-induced convulsions is further observed using disulfiram, which is an inhibitor of dopamine-β-hydroxylase and thus decreases brain NA and increases DA (27). The decrease in NA content may be involved in these alterations in the incidence of lidocaine- and PTZ-convulsions by disulfiram because disulfiram had similar opposite effects on lidocaine- and PTZ-convulsions as α-MPT, which decreased both amines, and L-DOPA, which increased DA content, rather increased the incidence of lidocaine-convulsions. Suenga et al. also studied the correlation between brain catecholamine level and post-decapitation convulsions in rats and showed that the depletion of central NA levels, especially in the spinal cord, was correlated with the reduction of duration in the clonic convulsions (28). It may be suggested from these results that the decrease of NA lowers the incidence of lidocaine-induced convulsions. Also the converse control by manipulation of brain amines of lidocaine- and PTZ-induced convulsions might be due to the difference in the mechanism(s) by which local anesthetic and PTZ produce convulsions. Ciarlone and Smudski reported that DA content was depleted in the mesencephalon-diencephalon using convulsive doses of lidocaine (21). They also reported that depletion of DA by the combined use of α-MPT and dihydroxyphenylserine lowered the threshold for lidocaine- and procaine-convulsions in rats (20), and concurrent depletion of NA and DA produced by α-MPT had no effect on the lidocaine seizure threshold (21). These findings are not consistent with the present findings that the decrease in brain NA or DA content resulted in the rise in the threshold for lidocaine-induced convulsions in mice. Whether this discrepancy is due to a species difference or other reasons is not known. Studies on the regional changes of brain amine content will be further required. It is well-known that sympathomimetic amines such as amphetamine and desipramine exert their CNS stimulating action by enhancing the release or inhibiting reuptake of biogenic amines. Increase of the incidence of lidocaine-convulsions by sympathomimetic amines may further support the hypothesis that depression of noradrenergic and/or dopaminergic neurons decreases the incidence of lidocaine-induced convulsions and stimulation of these neurons increases the incidence of convulsions. It is well-known that the disturbance of the function of GABAergic neurons with
GABA biosynthesis inhibitors or GABA receptor antagonists results in the development of convulsions. Thus, the inhibitory effects of local anesthetics on the GABA system as shown in the previous report (8) may be causally involved in the mechanism of local anesthetic-induced convulsions. On the other hand, the present results suggest that catecholaminergic systems may have an excitatory influence on lidocaine-induced convulsions. It has been reported that DA attenuates the effects of GABA on substantia nigra pars reticulata neurons (29). It may be possible that catecholaminergic neurons via the interneuronal connections to GABA neurons modulate lidocaine-convulsions. The amnergic control of lidocaine-induced convulsions may differ from the modulation of PTZ-induced convulsions.

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