Cerebral Monoamines and Lidocaine Toxicity in Rats

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The effect of alterations in whole brain monoamine content on the plasma lidocaine concentration resulting in seizures was studied in rats. Reductions in brain monoamine content were produced by treatment with one of the following drugs: reserpine, parachlorophenylalanine (PCPA), or alpha methyl paratyrosine (AMPT). Reserpine depleted norepinephrine (NE), and dopamine (DA) by 75 per cent and serotonin (5HT) by 54 per cent; PCPA reduced brain 5HT 56 per cent without changing NE and DA; AMPT reduced brain NE and DA by 54 and 60 per cent, respectively, without altering 5HT content. Treatment with 5-hydroxytryptophan, a serotonin precursor, combined with the peripheral decarboxylase inhibitor RO4-4602 increased brain 5HT content by 400 per cent without changes in DA and NE. Whole brain NE concentrations were assayed fluorometrically, DA brain concentrations were assayed by HPLC, and lidocaine concentrations in plasma were determined by gas chromatography. Plasma lidocaine concentrations at the onset of convulsions were found to be elevated significantly only by drugs causing serotonin depletion; increasing to 128 per cent of control with reserpine treatment and 139 per cent of control with PCPA treatment. Depletion of NE and DA had no effect on the lidocaine seizure threshold. Increases in brain 5HT caused a small but statistically significant decrease to 94 per cent of control in the mean plasma lidocaine concentration at seizure onset. (Key words: Anesthetics, local; lidocaine. Brain: convulsions; monoamines; seizure threshold. Neurotransmitters: dopamine, norepinephrine, serotonin. Toxicity: convulsions.)

LIDOCAINE-INDUCED SEIZURES are reported to originate within the amygdala and hippocampus.¹ ² ³ These areas of the limbic system appear to be innervated in part by monoaminergic neurons which utilize norepinephrine (NE), dopamine (DA), and serotonin (5HT) as neurotransmitters.³ ⁴ Using dose-response data deOliveira et al.⁵ ⁶ reported altered toxicity of lidocaine following drug treatments to decrease or increase brain serotonin content. The peripheral effects of catecholamines, however, also produce changes in hepatic blood flow and hepatic extraction of lidocaine. Lidocaine blood concentration during a constant infusion of lidocaine was observed to increase by 20 per cent with NE administration and decrease by 30 per cent with the administration of isoproterenol.⁷ Since lidocaine is cleared rapidly from blood during a first pass through the liver, the dose-response data may reflect alterations in hepatic removal with drug treatments rather than alterations in CNS toxicity of lidocaine. The measurement of plasma levels of lidocaine in blood perfusing the brain offers a more direct assessment of CNS sensitivity to lidocaine. This study investigates the plasma levels of lidocaine required to produce seizures in the presence of alterations in whole brain content of NE, DA, and 5HT.

Methods

Male Sprague Dawley rats weighing 250–400 g were assigned randomly to a control or drug treatment subgroup. On the appropriate day of the study, rats from each subgroup (control or experimental) were challenged with lidocaine at the same time. Lidocaine was injected in doses of 300–500 mg subcutaneously in the hind legs in order to produce a slowly rising blood concentration of lidocaine. Administration of lidocaine resulted in sedation of the rats before seizure onset. Animals which appeared to awaken from the sedative effects of lidocaine were redosed with lidocaine until seizures ensued. At the onset of generalized motor seizures, the rats were placed in a chamber containing a halothane saturated sponge. In approximately 10–20 s, apnea occurred and heart blood was drawn for the assay of the plasma concentration of lidocaine and its metabolite, MEGX, using a gas chromatographic method.⁸ Rats convulsing within two minutes of injection were excluded from this part of the study to avoid possible errors associated with rapidly rising blood levels of lidocaine that might not accurately reflect CNS lidocaine concentration. Plasma from heart blood (taken primarily from the left atrium) is equivalent to that entering the arterial circulation and is therefore considered to have a lidocaine concentration equal to that perfusing the brain.

CNS monoamine content was modified with drug treatments summarized in table 1. To deplete all three monoamines NE, 5HT, and DA, rats were treated with 1 mg/kg reserpine intraperitoneally on each of two days prior to the lidocaine seizure challenge.⁹ To deplete NE and DA with sparing of 5HT, alpha methyl paratyrosine, methyl ester, 300 mg/kg (AMPT), an inhibitor of tyrosine hydroxylase,¹⁰ was administered intraperitoneally four hours prior to the administration of lidocaine. To deplete 5HT with relative sparing of NE and DA, (PCPA) 350 mg/kg parachlorophenylalanine methyl ester, a tryptophanhydroxylase inhibitor¹⁰ was admin-
istered intraperitoneally 16–18 h prior to the lidocaine challenge. To increase intracerebral 5HT, rats were treated first with a peripheral decarboxylase inhibitor, RO4-4602 (RO), 50 mg/kg intraperitoneally followed in 30 min by a serotonin precursor 5-hydroxytryptophan (5-HTP), 100 mg/kg, intraperitoneally. Lidocaine was given to induce seizures four hours after giving 5HTP. This combination of drugs was used rather than the precursor 5HTP alone, in order to minimize peripheral consumption of the precursor. This permits more 5HTP to gain access to the intraneuronal space and to be converted to serotonin.

Brain 5HT was assayed using the fluorometric method of Maikel and Miller. Briefly, the technique uses homogenates in 10 volumes of acidified N-butanol, and an aqueous extract from the supernatant which was reacted with O-phthalaldehyde to form a fluorescent product. Brain NE was determined fluorometrically after purification and conversion to the trihydroxyindole derivative by the method of Robinson and Watts and brain DA was measured by HPLC with electrochemical detection. Brain content of 5HT, NE, and DA were determined in representative animals in each group. Since brain 5HT content was a reflection of the drug pretreatment regimen, the brain 5HT content of all animals receiving PCPA or 5HTP treatments were measured including those animals developing seizures in less than two minutes after lidocaine administration. Statistical significance between controls and experimental groups was determined using analysis of variance.

### Table 2. Results of Drug Treatment of Brain Monoamines

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>N</th>
<th>5HT (µg/g ± SEM)</th>
<th>NE (µg/g ± SEM)</th>
<th>DA (µg/g ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>0.76 ± 0.20</td>
<td>0.61 ± 0.05</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>Reserpine</td>
<td>8</td>
<td>0.35 ± 0.05*</td>
<td>0.14 ± 0.02*</td>
<td>0.06 ± 0.01*</td>
</tr>
<tr>
<td>AMPT</td>
<td>6</td>
<td>0.79 ± 0.35</td>
<td>0.26 ± 0.05*</td>
<td>0.10 ± 0.03*</td>
</tr>
<tr>
<td>PCPA</td>
<td>11</td>
<td>0.35 ± 0.17*</td>
<td>0.58 ± 0.01</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>5HTP plus RO4-4602</td>
<td>19</td>
<td>3.31 ± 2.52*</td>
<td>0.60 ± 0.09</td>
<td>0.27 ± 0.03</td>
</tr>
</tbody>
</table>

* *P* < 0.05.

### Results

Reserpine, a nonspecific depletor of brain monoamines, reduced NE and DA brain content by 75 per cent and 5HT brain content by 54 per cent (table 2). This treatment caused a significant increase to 128 per cent of control (*P* < 0.01) in the plasma lidocaine concentration at seizure onset (table 3). A similar decrease (55 per cent) in 5HT brain content was achieved by PCPA, an inhibitor of serotonin synthesis with little effect on NE and DA brain levels. The PCPA treatment caused an increase to 139 per cent of control in the plasma lidocaine concentration required to induce seizures (*P* < 0.05).

Increases in brain 5HT were achieved by treatment with 5HTP and RO. The mean brain content of 5HT was increased by more than 400 per cent by this treatment. Such treatment resulted in a small but not statistically significant decrease in the mean plasma lidocaine concentration at seizure onset (94 per cent of control). Subdividing the animals with increased 5HT content into two subgroups in which there was a 2- or 5-fold increase in 5HT resulted in no significant alteration in the lidocaine seizure threshold either between the two subgroups or from the control group. Treatment with AMPT depleted NE and DA content by 54 and 60 per cent, respectively, without change in 5HT content, resulted in no change in the mean plasma lidocaine concentration at seizure onset.

Mesoxyethylcinelexylidide (MEGX), the first metabolite formed in the degradation of lidocaine has also been reported to be equipotent to lidocaine in producing seizures. The concentration of this metabolite at the onset of seizures is shown on table 3. A significant decrease in MEGX (*P* < 0.05) was seen only with 5HTP treatment. MEGX tended to be higher but not statistically significant, with reserpine and AMPT treatment. Comparison of the sum of the lidocaine and MEGX present in the control and experimental groups did not alter the statistical significance observed with lidocaine alone on the seizure threshold with any of the treatments.

### Table 3. The Plasma Concentration of Lidocaine and MEGX at Onset of Seizure ± SEM

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Lidocaine (µg/ml)</th>
<th>MEGX (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.14 ± 0.61</td>
<td>1.63 ± 0.23</td>
</tr>
<tr>
<td>(n = 35)</td>
<td></td>
<td>(n = 29)</td>
</tr>
<tr>
<td>Reserpine</td>
<td>24.54 ± 0.90*</td>
<td>2.21 ± 0.94</td>
</tr>
<tr>
<td>(n = 13)</td>
<td></td>
<td>(n = 10)</td>
</tr>
<tr>
<td>PCPA</td>
<td>25.43 ± 2.27*</td>
<td>1.07 ± 0.18</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td>(n = 10)</td>
</tr>
<tr>
<td>AMPT</td>
<td>19.45 ± 0.86</td>
<td>2.22 ± 0.55</td>
</tr>
<tr>
<td>(n = 13)</td>
<td></td>
<td>(n = 13)</td>
</tr>
<tr>
<td>5HTP plus RO</td>
<td>17.96 ± 0.91</td>
<td>0.75 ± 0.08*</td>
</tr>
<tr>
<td>(n = 11)</td>
<td></td>
<td>(n = 11)</td>
</tr>
</tbody>
</table>

* *P* < 0.05.
Some behavioral differences associated with the drug pretreatments were observed. Animals treated with reserpine or AMPT were the most sedate and had the same requirement for lidocaine redosing prior to seizure onset as the control group. Animals treated with 5HTP appeared hyperactive, recovering rapidly from initial doses of lidocaine and consistently required (10 out of 11 animals) redosing with lidocaine prior to seizure onset. In contrast 20 of 35 in the control group were redosed with lidocaine before seizure onset.

Discussion

The results obtained in this study suggest that brain serotonin plays an important role in modulating lidocaine-induced seizures. The observation that pretreatment with reserpine (with lowering of all three monoamines) resulted in a significant increase in the lidocaine concentration at the onset of seizures, suggests that an alteration in one or all of the biogenic amines can alter the sensitivity to lidocaine-induced seizures. A similar change in lidocaine concentration necessary to induce seizures following PCPA and no change following AMPT treatment is consistent with serotonin being the primary biogenic amine modulating the lidocaine induced seizures. These results agree with the observation of deOliveira and Bretas who found the mean convulsant dose for lidocaine increased following PCPA treatment. In contrast, in this study, increasing brain serotonin levels with 5HTP failed to significantly alter the plasma lidocaine concentration required to induce seizures. A decrease in the plasma lidocaine concentration required to induce seizures was predicted by the observations of deOliveira et al. that increased brain serotonin lowered by almost 20 per cent the dose required to induce lidocaine seizures in mice. The difference in results may reflect species differences or altered lidocaine metabolism secondary to increased 5HT. Lidocaine is metabolized by the liver and its clearance approaches hepatic blood flow. Lidocaine blood concentration has been observed to increase 20 per cent during a constant lidocaine infusion with the administration of norepinephrine and to decrease 30 per cent with the administration of isoproterenol. While data on the effect of 5HT (or 5HTP) on lidocaine removal or hepatic blood flow is unknown, it is feasible that previous reports of a lowered lidocaine seizure threshold and prolonged seizures with increased 5HT was the result of achieving a higher blood level of drug secondary to decreased removal of lidocaine by the liver after 5HTP pretreatment. The prolongation of lidocaine seizure caused by 5HTP pretreatment may have also been the result of decreased brain lidocaine efflux. Decreased efflux from brain of lidocaine has been observed with iproniazid treatment.

Fundamental differences exist between lidocaine-induced seizures and experimental seizures produced by audiogenic, electroshock, and pentylentetrazol administration. Depletion of brain serotonin increases brain susceptibility to these three modes of producing seizure activity while protecting against seizures induced by lidocaine. In contrast increases in brain 5HT content protects against audiogenic and pentylentetrazol seizures but in this study had little effect on lidocaine blood levels needed to produce seizures.

Since lidocaine seizures appear to originate in the limbic system (amygdala and hippocampus) while the other modes of producing seizures originate outside of the limbic system, the effect of altering 5HT may produce different results. Serotonin appears to function primarily as an inhibitory transmitter substance with inhibitory effects being mediated by both pre- and postsynaptic 5HT receptors in the CNS. Microiontophoretic application of serotonin into specific brain regions may cause either inhibition or stimulation of spontaneous firing rates depending on what region is tested and on the sensitivity of the pre- and postsynaptic receptor. Although the major effect of 5HT on postsynaptic 5HT receptors is inhibition of neuronal firing, an increase in the spontaneous firing rate following the microiontophoretic application of 5HT has been observed. This could represent disinhibition via a presynaptic action of an inhibitory serotonergic neuron synapsing on an excitatory neuron, with a net increase in regional neuronal excitability. Alternately, 5HT could also produce a net increase in neuronal excitability by acting on postsynaptic receptors located on neurons which in turn synapse on other inhibitory neurons. A similar disinhibition of excitatory pathways would result. Such mechanisms in the serotonin-rich amygdala may result in increased neuronal activity and facilitate spread of seizure discharges induced by lidocaine. In contrast, other forms of experimental seizures may be originated in brain regions where 5HT results in a net decrease in excitability.

While extrapolation of results in rats to humans is hazardous, this study suggests several potential clinical implications. Most apparent is the possibility that patients being treated with reserpine may have a degree of protection against lidocaine-induced seizures. Likewise, other drugs such as tricyclic antidepressants and the appetite suppressant fenfluramine which may cause depletion of brain 5HT, might be expected to increase the seizure threshold to lidocaine. Furthermore, patients who are users of the antagonists of 5HT such as ergot derivatives or lysergic acids derivatives (LSD) may be protected against lidocaine-induced seizures. In contrast, patients taking medications which alter norepinephrine or dopamine brain content such alphamethylldopa or DOPA (including those with decarboxylase inhibitors)
would not be expected to have altered CNS local anesthetic sensitivity.

References