Elevation of \(\gamma\)-Aminobutyric Acid in Brain: Selective Inhibition of \(\gamma\)-Aminobutyric-\(\alpha\)-ketoglutaric Acid Transaminase*

CLAUDE F. BAXTER AND EUGENE ROBERTS

From the Department of Biochemistry, Medical Research Institute, City of Hope Medical Center, Duarte, California

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The quantitative measurement of amino acids in different areas of the central nervous system has shown that a progressive increase takes place in levels of \(\gamma\)-aminobutyric acid during development (2, 3), and that the concentration of this amino acid in adult brain is relatively constant under a variety of physiological stresses. \(\gamma\)-Aminobutyric acid is synthesized by glutamic acid decarboxylase, an enzyme unique to tissues of the central nervous system (4), and is degraded by \(\gamma\)-aminobutyric acid-\(\alpha\)-ketoglutaric acid transaminase, which is found also in other tissues of mammals (5). Both of these enzymes require pyridoxal-P as a cofactor (4, 6). It has been shown that, under normal conditions, the level of \(\gamma\)-aminobutyric acid in any particular brain area is directly related to levels of maximal potential glutamic acid decarboxylase activity (7), but appears unrelated to levels of \(\gamma\)-aminobutyric acid-\(\alpha\)-ketoglutaric acid transaminase.1

The concentration of \(\gamma\)-aminobutyric acid in the brains of adult rodents is altered after the intraperitoneal or intravenous injection of a number of compounds (8-14). Among the carbonyl trapping agents are compounds which elevate or depress levels of \(\gamma\)-aminobutyric acid. Thiosemicarbazide, injected into rats, lowers levels of \(\gamma\)-aminobutyric acid and decreases glutamic acid decarboxylase activity (7), whereas hydroxylamine and aminoxyacetic acid elevate \(\gamma\)-aminobutyric acid in the brains of rats (9, 14).

The present communication describes some quantitative and temporal details of the interrelationship found between the levels of \(\gamma\)-aminobutyric acid, and the activities of the enzymes involved in the synthesis and destruction of this amino acid, when hydroxylamine or aminoxyacetic acid was administered to animals. Results of these experiments, which show that these compounds inhibit \(\gamma\)-aminobutyric acid-\(\alpha\)-ketoglutaric acid transaminase in brain, are in agreement with the hypothesis that the selective elevation of \(\gamma\)-aminobutyric acid in brain tissue after administration of hydroxylamine in vivo is the result of a decreased rate of utilization of \(\gamma\)-aminobutyric acid by the transaminase reaction at a time when formation by the glutamic acid decarboxylase pathway continues relatively uninterrupted (10).

EXPERIMENTAL PROCEDURE

Animals and Brain Samples—Adult Sprague-Dawley rats were used. Brain areas for the assay of \(\gamma\)-aminobutyric acid were sampled and analyzed as described previously (15). Samples of cortex were taken from nonhomologous loci of each hemisphere of the brains of monkeys and cats before treatment with hydroxylamine. Areas of cortex which were homologous to the control samples were excised after treatment with hydroxylamine. In these experiments, each animal served as its own control. This technique has been described previously for the cat (16).

Treatment—Carbonyl trapping agents and pyridoxal were administered intraperitoneally to cats and monkeys and intravenously to rats. Solutions of hydroxylamine hydrochloride and aminoxyacetic acid hemihydrochloride were prepared fresh just before use, and neutralized with sodium hydroxide to pH 6 or 6.5 before being injected into animals.

Analysis—\(\gamma\)-Aminobutyric acid was determined in brain tissue extracts enzymatically as previously described (10, 15). Glutamic acid decarboxylase activity in brain homogenates was measured by the determination of the increase in the amount of \(\gamma\)-aminobutyric acid during incubation of brain homogenates with glutamic acid. The system was similar to that employed previously for the assay of glutamic acid decarboxylase (4). The incubation mixture consisted of 30 mg fresh weight of brain homogenate, 10.3 mg of glutamic acid (neutralized) and 50 \(\mu\)g of pyridoxal-P (when indicated), in a final volume of 0.6 ml of 0.1 M phosphate buffer, pH 6.3. Samples were incubated in closed vials at 37° with shaking. In contrast to the glutamic acid decarboxylase activity in extracts of brain acetone powder, the activity of the enzyme in the homogenate system was not inhibited significantly by aerobic incubation conditions. Vials containing pyridoxal-P were incubated for 30 minutes while those with no pyridoxal-P addition were incubated for shorter periods, the activity being expressed in all instances as micrograms of product formed per g of tissue per hour. Variations in this protocol are noted with the pertinent experiments. At the end of the incubation period, the reaction was stopped by addition of 3 ml of 95% ethyl alcohol, and samples were assayed for \(\gamma\)-aminobutyric acid in the usual manner. Corrections for initial content of \(\gamma\)-aminobutyric acid were obtained by measuring the levels found at zero time or after incubation of homogenate without glutamic acid substrate. Both methods gave essentially the same “blank” value. All assays were run in duplicate. Results obtained for glutamic acid decarboxylase activity by this method were in general agreement with results obtained for the same enzymatic activity by measuring the \(\text{C}^{4}\text{O}_{2}\) evolution from \(\text{C}^{4}\)-labeled glutamic acid (17).

\(\gamma\)-Aminobutyric acid-\(\alpha\)-ketoglutaric acid transaminase activity

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* D. G. Simonsen and E. Roberts, manuscript in preparation.
Glutamic acid, and pyrrolidone carboxylic hydroxamide were N-lauroyl y-aminobutyric acid, y-ethylglutamic acid, y-methyl-butyric acid isopropyl ester, N-acetyl y-aminobutyric acid, Chemical Division of Eastman Kodak. The N-methyl derivative of hydroxylamine was synthesized from nitromethane by the method described by Weygand (19). y-Aminobutyric acid hydroxamic acid, y-aminobutyric acid ethyl ester, y-aminoacetylmonoxime, butyrolactone, nitromethane, phenylhydrazine, derivative of hydroxylamine, as well as sodium malonate, diaretanilide, and p-aminopropiophenone were obtained from the ascending two-dimensional chromatographic procedures based on the enzymatic assay of y-aminobutyric acid. The extracts of enzyme in extracts of beef brain acetone powders (6). The injected dose is expressed as free base.

**TABLE I**

Effect of hydroxylamine on y-aminobutyric acid levels in cortex of different species

<table>
<thead>
<tr>
<th>Specie</th>
<th>Body weight</th>
<th>Hydroxylamine</th>
<th>Control y-aminobutyric acid level</th>
<th>Treated y-aminobutyric acid level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus monkey</td>
<td>7.8 kg</td>
<td>7.4 mg/kg</td>
<td>14</td>
<td>37</td>
</tr>
<tr>
<td>Cat</td>
<td>2.5 kg</td>
<td>9.6 mg/kg</td>
<td>20</td>
<td>43</td>
</tr>
<tr>
<td>Sprague-Dawley rat</td>
<td>0.2 kg</td>
<td>37.0 mg/kg</td>
<td>20</td>
<td>54</td>
</tr>
<tr>
<td>Swiss mouse</td>
<td>0.02 kg</td>
<td>50.0 mg/kg</td>
<td>27</td>
<td>23</td>
</tr>
</tbody>
</table>

* The levels of y-aminobutyric acid are expressed as micrograms per 100 mg of fresh brain tissue. Recorded values are averages of individual measurements in brain tissues of 3 monkeys, 5 cats, 5 mice, and 36 rats. The largest deviation from any single value from the average of a group was no greater than 20%.

† The injected dose is expressed as free base.

was determined by a method devised for measurement of this enzyme in extracts of beef brain acetone powders (6). The equivalent of 60 mg of homogenized rat brain in 0.1 M borate buffer, pH 8.2, was incubated for 1 hour with a-ketoglutarate and y-aminobutyric acid substrate at 37°. No pyridoxal-P was added to the mixture unless specifically indicated. Homogenates incubated in a system from which a-ketoglutarate was omitted served as blanks.

Tissue extracts were prepared for chromatography by a method identical to that used in the preparation of samples for the enzymatic assay of y-aminobutyric acid. The extracts of 30 mg of fresh tissue contained in 50 µl were analyzed by descending two-dimensional chromatographic procedures based upon well established techniques (18).

**Materials—** Hydroxylamine, mercaptoethanol and the O-methyl derivative of hydroxylamine, as well as sodium malonate, diacetylmonoamide, butyrolactone, nitromethane, phenylhydrazine, acetonilide, and p-aminopropiophenone were obtained from the Chemical Division of Eastman Kodak. The N-methyl derivative of hydroxylamine was synthesized from nitromethane by the method described by Weygand (19). γ-Aminobutyric acid hydroxamic acid, γ-aminobutyric acid ethyl ester, γ-aminobutyric acid isopropyl ester, N-acetyl γ-aminobutyric acid, N-lauroyl γ-aminobutyric acid, γ-ethylglutamic acid, γ-methylglutamic acid, and pyrrolidine carboxylic hydroxamic acid were donated by Dr John R. Sunnygard of General Mills, Inc. Glutamic acid, L-tyrosine hydroxamide, and α-ketoglutaric acid were purchased from Sigma Chemical Company, and sodium barbiturate from Mallinckrodt Chemical Works. All salts, sodium arsenite, and sodium nitrite were reagent grade chemicals purchased from J. T. Baker Chemical Company.

**RESULTS**

Levels of γ-Aminobutyric Acid after Hydroxylamine Administration—Increases in levels of γ-aminobutyric acid were found in all areas of brain examined after injection of hydroxylamine into cats, rats, and monkeys. Of the species studied to date, only the mouse has failed to respond to hydroxylamine with elevated levels of γ-aminobutyric acid in the brain. The small but consistent decrease in levels of γ-aminobutyric acid in the mouse cortex after treatment with hydroxylamine is illustrated in Table I.

In the rat, the extent of elevation of γ-aminobutyric acid differed for each brain area (10). Time and dosage response curves for four of these areas are illustrated in Figs. 1 and 2. After the intraperitoneal administration of 75 mg of hydroxylamine hydrochloride per kg of body weight to rats, the level of γ-aminobutyric acid in all tested areas of the brain started to rise within 30 minutes and reached a peak approximately 90 minutes after injection. Elevated levels of γ-aminobutyric acid persisted in some areas for more than 24 hours (Fig. 1).

Each area of brain appears to have characteristic levels of glutamic acid decarboxylase and γ-aminobutyric acid-α-keto-glutaric acid transaminase activities (20-22). Levels of γ-aminobutyric acid were measured in representative areas with a combination of high decarboxylase and high transaminase (cerebellum), and low decarboxylase and low transaminase (cortex) at 90 minutes after intraperitoneal injection of various doses of hydroxylamine hydrochloride. Those areas with high glutamic acid decarboxylase levels showed a quantitatively greater increase of γ-aminobutyric acid levels with increasing dosage than did those in which the level of decarboxylase activity was low (Fig. 2).

Effect of Methemoglobinemia—A well known effect of hydroxylamine, when injected into mammals, is the formation of methemoglobin (23). In young adult rats, an injection of hydroxylamine hydrochloride at a level of 75 mg per kg of body weight resulted in an initial blanching and subsequent darkening of the extremities within 4 to 10 minutes. Occasionally, a mild convulsion caused by temporary anoxia occurred within 6 minutes. Animals were prostrated for about 30 minutes and then recovered rapidly. The blood of many of the hydroxylamine treated rats contained visible amounts of methemoglobin at the time of decapitation. The possibility that methemoglobin and the resulting anoxia were responsible for elevated levels of γ-aminobutyric acid in brain was checked. Several substances known to produce methemoglobinemia (28) were injected into rats. Visible darkening of the blood was produced by all of the agents except acenilide. In no instance was the formation of methemoglobin (and the concomitant anoxia) accompanied by an elevation in the levels of γ-aminobutyric acid in any area of the rat brain (Table II). Methemoglobinemia per se, therefore, can be eliminated as a primary cause of the elevated levels of γ-aminobutyric acid.

Hydroxylamine is metabolized to ammonia by the hemoglobin of human blood (24). However, the administration of ammonium salts produced no elevation of γ-aminobutyric acid in...
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C. F. Baxter and E. Roberts

CORPORA QUADRIGEMINA

CORTEX

CEREBELLUM

20 25 30 35

HOURS

FIG. 1. Changes in levels of \( \gamma \)-aminobutyric acid (\( \gamma \)-ABA) in three areas of rat brain after treatment with hydroxylamine. Response in time to injection of 0.5 to 0.7 ml of hydroxylamine hydrochloride. Injected dose, 75 mg per kg of body weight. Rats were decapitated at various times after injection. Range of results with 3 to 5 animals per time period is indicated. The best curve was drawn through averages. The range of normal values for each brain area, based upon individual determinations from more than three dozen rats, is indicated by shading.

brains of rats\(^1\) or in dogs (25) as evaluated by chromatographic analysis.

**Specificity of Hydroxylamine in Vivo**—A survey of derivatives of hydroxylamine and compounds related to \( \gamma \)-aminobutyric acid and glutamic acid showed that only hydroxylamine produced the elevation in levels of \( \gamma \)-aminobutyric acid in the brains of rats. A number of oximes and hydroxamides were tested because of the possibility that passage through the blood-brain barrier, and subsequent hydrolysis, might result in a slow intracerebral release of hydroxylamine. However, all of the substances tested failed to elevate levels of \( \gamma \)-aminobutyric acid in brain in vivo. The following compounds were tested: \( \alpha \)-oximinophenylpropionic acid (218 mg per kg), \( \alpha \)-oximinobenzylpropionic acid (236 mg per kg), \( \alpha \)-oximinocaproic acid (156 mg per kg), diacetelymonoxime (150 mg per kg), L-tyrosine hydroxamide (211 mg per kg), \( \gamma \)-aminobutyric acid hydroxamide (125 mg per kg), isonicotinyl hydroxamide (149 mg per kg), pyrrolidone carboxylic hydroxamide (156 mg per kg), \( \gamma \)-aminobutyric acid ethyl ester (300 mg per kg), and \( \gamma \)-aminobutyric acid isopropyl ester monohydrochloride (500 mg per kg). The last named compound was extremely toxic. \( \gamma \)-Aminobutyric acid and glutamic acid derivatives, \( N \)-acetyl \( \gamma \)-aminobutyric acid (2 g per kg), \( N \)-lauroyl \( \gamma \)-aminobutyric acid (2 g per kg), \( \gamma \)-ethylglutamic acid (750 mg per kg) and \( \gamma \)-methylglutamic acid (500 mg per kg), were thought to pass with greater ease through the blood-brain barrier. All of these compounds were ineffective in changing levels of \( \gamma \)-aminobutyric acid in brain. \( N \)-Lauroyl \( \gamma \)-aminobutyric acid was extremely toxic.

Experiments with \( N \)-Methyl and \( O \)-Methyl Hydroxylamine—The relative importance of the amino and hydroxy group of hydroxylamine in the inhibition of \( \gamma \)-aminobutyric acid-\( \alpha \)-ketoglutaric acid transaminase was investigated. \( O \)-Methyl hydroxylamine had no effect in vivo upon the level of \( \gamma \)-aminobutyric acid in rat brain, but inhibited the transaminase when added directly to a homogenate system. This inhibition was weaker than that produced by hydroxylamine in equimolar amounts. \( N \)-Methyl hydroxylamine was inactive both in vivo and in vitro (Table III). The requirement in vitro of an intact amino group is in agreement with the hypothesis that hydroxylamine interacts with the aldehyde group of the pyridoxal-P coenzyme of \( \gamma \)-aminobutyric acid-\( \alpha \)-ketoglutaric acid transaminase. \( O \)-Methyl hydroxylamine may be inactive in vivo because of its inability to pass the blood-brain barrier.

**Inhibition of \( \gamma \)-Aminobutyric Acid-\( \alpha \)-Ketoglutaric Acid Transaminase by Hydroxylamine**—Evidence is presented in Table IV that hydroxylamine administered in vivo inhibits the transaminase directly. Enzyme assays of \( \gamma \)-aminobutyric acid-\( \alpha \)-ketoglutaric acid transaminase in brain tissue of normal and hydroxylamine-treated animals indicated that, in the latter, the transaminase was inhibited by more than 50%. Injection of pyridoxine or pyridoxal into hydroxylamine-treated rats did not reverse this
inhibition of the transaminase by hydroxylamine and more effectively by the addition of pyridoxal-5-phosphate in two groups of four animals each. Levels of α-aminobutyric acid are expressed as micrograms per 100 mg of fresh brain tissue. After 90 minutes, the time of greatest inhibition of the transaminase, the enzyme system with hydroxylamine could be partially reversed to the incubation mixture (6). Reactivation with pyridoxal-5-phosphate indicated that the inhibition in vitro of the α-aminobutyric acid-α-ketoglutaric acid transaminase activity in vivo may proceed by a mechanism which differs from that observed in vitro.

Structural elements and membrane barriers of brain tissue may be altered or destroyed during homogenization, and eliminated in extracts of acetone powder preparations. The differences in effect of inhibitors and activators of an enzyme system in intact brains of rats in vivo may be attributed to changes. Alternatively, the results suggest the possibility that hydroxylamine inhibition of α-aminobutyric acid-α-ketoglutaric acid transaminase in vivo may proceed by a mechanism which differs from that observed in vitro.

Comparison of Treatment with Hydroxylamine and Aminooxyacetic Acid—Both hydroxylamine and aminooxyacetic acid have been shown to elevate levels of α-aminobutyric acid in brains of rats in vivo (10, 14). Experiments in vitro have demonstrated that these reagents are powerful inhibitors at low concentrations of both α-aminobutyric acid-α-ketoglutaric acid transaminase and glutamic acid decarboxylase (6, 26). In contrast, aminooxyacetic acid has no demonstrable effect upon glutamic acid decarboxylase in vivo (Fig. 5). Under similar conditions, hydroxylamine had only a slight inhibitory effect upon this enzyme (Table IV, Fig. 4).

The changes in levels of α-aminobutyric acid, and in the activities of the transaminase and decarboxylase in whole rat brain after the injection of hydroxylamine or aminooxyacetic acid, were studied as a function of time. Results are shown in Figs. 4 and 5. Although both compounds produced similar results, aminooxyacetic acid appears to have the more powerful and prolonged effect. In both instances, the time of maximal elevation of α-aminobutyric acid corresponded closely to the time of greatest inhibition of the transaminase. At later time periods, levels of α-aminobutyric acid returned to normal more rapidly than the transaminase activity. One possibility suggested by this discrepancy is that an alternate pathway for the utilization of α-aminobutyric acid may be facilitated when the transaminase, the primary pathway, is inhibited. This possibility is under investigation.

When both hydroxylamine and aminooxyacetic acid were injected into the same animal, the inhibition of the α-aminobutyric acid-α-ketoglutaric acid transaminase approached the sum of the inhibitions shown with either substance alone (Table VI). Periods beyond 1½ hours were not studied since in the doses employed the combination of drugs was lethal within 2½ hours.

Accumulation of β-Alanine in Liver—In addition to their effect upon α-aminobutyric acid-α-ketoglutaric acid transaminase of

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**Table II**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg body weight)</th>
<th>Cortex</th>
<th>Cerebellum</th>
<th>Colliculi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>26 (23-29)</td>
<td>28 (20-34)</td>
<td>56 (48-61)</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>180</td>
<td>26 (22-29)</td>
<td>30 (20-31)</td>
<td>57 (54-58)</td>
</tr>
<tr>
<td>Acetanilide</td>
<td>150</td>
<td>24 (22-29)</td>
<td>29 (25-29)</td>
<td>45 (44-47)</td>
</tr>
<tr>
<td>Aminopropiophenone</td>
<td>40</td>
<td>24 (23-26)</td>
<td>26 (24-27)</td>
<td>50 (48-53)</td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>5</td>
<td>25 (23-27)</td>
<td>34 (33-35)</td>
<td>59 (58-64)</td>
</tr>
<tr>
<td>Hydroxylamine hydrochloride</td>
<td>75</td>
<td>47 (45-54)</td>
<td>38 (35-44)</td>
<td>85 (77-95)</td>
</tr>
</tbody>
</table>

* Hydroxylamine or the methylated derivatives were injected intraperitoneally at levels equivalent to 75 mg of hydroxylamine hydrochloride per kg of body weight. After 90 minutes, the levels of α-aminobutyric acid were determined in tissues of the cortex. The methyl derivatives of hydroxylamine were tested in two groups of four animals each. Levels of α-aminobutyric acid are expressed as micrograms per 100 mg of fresh brain tissue.

† A homogenate of normal rat brain was prepared and potential inhibitors were tested in vitro at final concentrations of 1 X 10⁻³ M. No pyridoxal-P was added. Activity is expressed as micromoles of glutamic acid formed per g of fresh tissue per hour.
TABLE IV
Effect of hydroxylamine and pyridoxal upon the levels of y-aminobutyric acid, y-aminobutyric acid-α-ketoglutaric acid transaminase, and glutamic acid decarboxylase in rat brain in vivo

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Rats tested*</th>
<th>Compounds injected†</th>
<th>Time after initial injection</th>
<th>Level of y-aminobutyric acid</th>
<th>y-Aminobutyric acid-α-ketoglutaric acid transaminase</th>
<th>Glutamic acid decarboxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>min</td>
<td>µε/100 mg fresh tissue</td>
<td>µmoles glutamic acid/g tissue/hr</td>
</tr>
<tr>
<td>Whole brain</td>
<td>2</td>
<td>None</td>
<td></td>
<td></td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>Whole brain</td>
<td>2</td>
<td>Hydroxylamine</td>
<td>75</td>
<td>36</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Whole brain</td>
<td>2</td>
<td>Hydroxylamine</td>
<td>210</td>
<td>48</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>4</td>
<td>None</td>
<td>90-100</td>
<td>29</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>4</td>
<td>Hydroxylamine</td>
<td></td>
<td>19</td>
<td>61</td>
<td>47</td>
</tr>
<tr>
<td>Cortex</td>
<td>5</td>
<td>None</td>
<td>90-100</td>
<td>21</td>
<td>44</td>
<td>33</td>
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<tr>
<td>Cortex</td>
<td>5</td>
<td>Hydroxylamine</td>
<td></td>
<td>21</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Cortex</td>
<td>2</td>
<td>Pyridoxal</td>
<td>90</td>
<td>45</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Cortex</td>
<td>2</td>
<td>Hydroxylamine and pyridoxal</td>
<td>90</td>
<td>40</td>
<td>41</td>
<td>38</td>
</tr>
<tr>
<td>Cortex</td>
<td>2</td>
<td>Hydroxylamine and pyridoxal</td>
<td>80</td>
<td>36</td>
<td>17</td>
<td>13</td>
</tr>
</tbody>
</table>

* Results are based upon experiments with female rats weighing 180 to 210 g.
† Hydroxylamine hydrochloride was injected at a level of 75 mg per kg of body weight. Pyridoxal hydrochloride (partially neutralized) was administered at a level of 400 mg per kg body weight.

brain, hydroxylamine and aminooxyacetic acid appear to bring about an accumulation of β-alanine in the liver. Presumably, this is the result of an inhibition of β-alanine-α-ketoglutaric acid transaminase, an enzyme that may be identical to the γ-aminobutyric-α-ketoglutaric acid transaminase of brain and other tissues (5, 27). Chromatographic evidence for the specificity of hydroxylamine and aminooxyacetic acid in brain and liver are shown in Fig. 6. Administration of hydroxylamine resulted in no significant changes in the level of ninhydrin-reactive constituents in liver and brain other than those already mentioned. In rats treated with aminooxyacetic acid, β-alanine accumulation was noted also in kidney and spleen. No changes were found in

![Graph](http://www.jbc.org/)

**Fig. 3.** Lineweaver-Burke plot of γ-aminobutyric acid-α-ketoglutaric acid transaminase activity as a function of substrate and inhibitor concentration. Technical difficulties prevented the testing of α-ketoglutarate as a competitive substrate. Hydroxylamine and γ-aminobutyric acid (γ-ABA) were added simultaneously to the incubation mixture.
Selective Inhibition of γ-Aminobutyric Acid Transaminase

Successful methods for elevating the levels of γ-aminobutyric acid in brain tissue in vivo can be divided into two groups. Some investigators have modified the blood-brain barrier by physical or chemical means, thereby permitting entry of exogenous γ-aminobutyric acid into brain tissue (37, 38). Alternatively, endogenous levels of γ-aminobutyric acid have been elevated, presumably by inhibiting a step in the reaction sequence involved in the degradation of γ-aminobutyric acid. Of the many compounds tested, only hydroxylamine, aminooxyacetic acid, and lethal doses of 2,4-diaminobutyric acid (11, 12) have been shown to elevate endogenous γ-aminobutyric acid. The report that ethanol administered in vivo elevated levels of γ-aminobutyric acid (39) could not be confirmed with fed Sprague-Dawley rats.

Hydroxylamine has been shown to elevate γ-aminobutyric acid, probably through the selective inhibition of γ-aminobutyric acid-α-ketoglutaric acid transaminase. If inhibition is attained in skeletal muscle, but in heart muscle a decrease in lysine content was observed which may be related to shifts in electrolyte balance. Levels of lysine have been shown to increase in muscle of potassium-deficient rats (28).

Failure of Dehydrogenase Inhibitors to Produce Increases in γ-Aminobutyric Acid in Vivo—Since the equilibrium of the isolated transamination reaction

\[ \text{NH}_2-(\text{CH}_2)_3-\text{COOH} + \text{COOH}-(\text{CH}_2)_3-\text{COOH} \rightleftharpoons \text{CHO}-(\text{CH}_2)_3-\text{COOH} + \text{COOH}-(\text{CH}_2)_3-\text{COOH} \]

is to be left (29) and is shifted to the right only when coupled to the enzymatic dehydrogenase reaction

\[ \text{CHO}-(\text{CH}_2)_3-\text{COOH} + 2\text{DPN} + \text{H}_2\text{O} \rightleftharpoons \text{COOH}-(\text{CH}_2)_3-\text{COOH} + 2\text{DPNH} \]

it appeared possible that hydroxylamine also might be elevating γ-aminobutyric acid by inhibiting the DPN-linked dehydrogenase reaction. Experiments conducted to explore the feasibility of elevating γ-aminobutyric acid through an inhibition of the DPN-linked succinic semialdehyde dehydrogenase in vivo were uniformly negative. The depression of DPN levels with 3-acylpyridine (160 mg per kg) (30), aminooxyacetic acid (75 mg per kg) (31), or the elevation of DPN levels with niacinamide (100 mg per kg) (32) failed to alter the level of γ-aminobutyric acid in rat brains. Similarly negative results were obtained with sodium oxamate (200 mg per kg), sodium barbiturate (150 mg per kg), with arsenite (5 mg per kg), another inhibitor of succinic semialdehyde dehydrogenase (33), and with sodium malonate (1 g per kg) (34), an inhibitor of succinate oxidation.

It has been shown that brain converts γ-hydroxybutyric acid (sodium salt) to succinic semialdehyde enzymatically (35). Although the intraperitoneal injection of this compound into rats had very profound physiological effects (36), it did not alter the levels of γ-aminobutyric acid in treated animals. All tests were conducted within 1 to 2½ hours after injection of these compounds.

**DISCUSSION**

Successful methods for elevating the levels of γ-aminobutyric acid in brain tissue in vivo can be divided into two groups. Some investigators have modified the blood-brain barrier by physical or chemical means, thereby permitting entry of exogenous γ-aminobutyric acid into brain tissue (37, 38). Alternatively, endogenous levels of γ-aminobutyric acid have been elevated, presumably by inhibiting a step in the reaction sequence involved in the degradation of γ-aminobutyric acid. Of the many compounds tested, only hydroxylamine, aminooxyacetic acid, and lethal doses of 2,4-diaminobutyric acid (11, 12) have been shown to elevate endogenous γ-aminobutyric acid. The report that ethanol administered in vivo elevated levels of γ-aminobutyric acid (39) could not be confirmed with fed Sprague-Dawley rats.

Hydroxylamine has been shown to elevate γ-aminobutyric acid, probably through the selective inhibition of γ-aminobutyric acid-α-ketoglutaric acid transaminase. If inhibition is attained

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**Fig. 4.** A comparison of responses to hydroxylamine of γ-aminobutyric acid levels, γ-aminobutyric acid-α-ketoglutaric acid transaminase activity and glutamic acid decarboxylase activity. Changes with time after injection of 0.5 to 0.7 ml of hydroxylamine hydrochloride. Injected dose, 76 mg per kg of body weight. The activities of the transaminase and the dehydrogenase were measured without the addition in vitro of pyridoxal-P. All activities and levels are those found in homogenates of whole brain without pons and medulla. The abbreviations used are: γ-ABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; γ-ABA-T, γ-aminobutyric acid-α-ketoglutaric acid transaminase.

**Fig. 5.** A comparison of responses to aminooxyacetic acid of γ-aminobutyric acid levels, γ-aminobutyric acid-α-ketoglutaric acid transaminase activity and glutamic acid decarboxylase activity. Changes with time after injection of solution of aminooxyacetic acid. Injected dose, 50 mg per kg of body weight. The activities of the transaminase and the dehydrogenase were measured without the addition in vitro of pyridoxal-P. All activities and levels are those found in homogenates of whole brain without pons and medulla. The abbreviations used are: γ-ABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; γ-ABA-T, γ-aminobutyric acid-α-ketoglutaric acid transaminase.
through the interaction of hydroxylamine with the aldehyde group of the pyridoxal-P coenzyme, the differential effect of hydroxylamine upon glutamic acid decarboxylase and the transaminase might be explained in terms of the relatively greater affinity of the pyridoxal-P oxime for the apoenzyme of the transaminase than for that of the decarboxylase. There is presumptive evidence that pyridoxal-P itself is more tightly bound to the transaminase than to the decarboxylase apoenzyme (40). It is conceivable that the transaminase-pyridoxal-P-oxime complex may be more stable than the decarboxylase complex, from which the inactive coenzyme might dissociate more easily with the consequent reactivation by pyridoxal-P available in the tissue. Recent results in our laboratory have shown that pyridoxal oxime does not inhibit the γ-aminobutyric acid-α-ketoglutaric acid transaminase in vivo! This observation is in accord with the concept that the apoenzyme-coenzyme bond of the transaminase is very strong, permitting little, if any, substitution with the oxime directly. It is of interest to note in this regard that glutamic acid decarboxylase was inhibited by pyridoxal oxime in these experiments. The competitive nature of the hydroxylamine inhibition in vitro (Fig. 3) suggests that the free aldehyde group on the coenzyme is a likely site of attachment for the substrate of the transaminase. A similar conclusion was reached in a study on inhibition of the bacterial glutamic acid decarboxylase by hydroxylamine (41). Although studies in vitro with glutamic acid decarboxylase and γ-aminobutyric acid-α-ketoglutaric acid transaminase from mammalian brain have shown that hydroxylamine and aminoxyactic acid are powerful inhibitors of both enzymes, in the intact animal only one of these enzymes is inactivated to an appreciable extent. These differences may be related to the interaction of the inhibitor with permeability and transport phenomena in the intact tissue. Also, the possibility must be considered that the injected inhibitor was changed in vivo in some way before exerting its biochemical effect.

SUMMARY

1. An elevation of levels of γ-aminobutyric acid in the brains of rats, cats, and monkeys was observed after the intraperitoneal injection of nonlethal doses of hydroxylamine. Of all species tested, only the mouse failed to respond to hydroxylamine in a similar manner.

2. The response to hydroxylamine was studied in rats as a function of dosage and time. The largest and most rapid elevation of γ-aminobutyric acid was noted in areas of brain which contain high levels of glutamic acid decarboxylase activity. Elevation of γ-aminobutyric acid persisted in some areas for as long as 24 hours.

3. The increase in γ-aminobutyric acid of brain tissue in vivo was not the result of methemoglobinemia, anoxia, or of ammonia intoxication.

4. Hydroxylamine and aminoxyactic acid inhibited γ-aminobutyric acid-α-ketoglutaric acid transaminase in vitro without appreciably affecting the activity of glutamic acid decarboxylase. This contrasts with in vitro studies in which both enzymes were inhibited by these compounds at low concentrations.

5. Aminoxyactic acid had a longer lasting effect than hydroxylamine upon the elevation of γ-aminobutyric acid in brain. Both compounds elevated levels of β-alanine in liver. Levels of γ-aminobutyric acid returned to normal more rapidly than did the activity of γ-aminobutyric acid-α-ketoglutaric acid transaminase after treatment with these drugs. Possible implications of this observation are discussed.

6. Hydroxylamine was shown to be a competitive inhibitor of γ-aminobutyric acid-α-ketoglutaric acid transaminase, probably by competing with the amino group of γ-aminobutyric acid for attachment to the aldehyde group of the coenzyme.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Injected dose</th>
<th>Inhibition after 15 min</th>
<th>Inhibition after 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>0</td>
</tr>
<tr>
<td>Hydroxylamine</td>
<td>75</td>
<td>40</td>
<td>59</td>
</tr>
<tr>
<td>Aminoxyactic acid</td>
<td>20</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Hydroxylamine and aminoxyactic acid</td>
<td>75 + 20</td>
<td>77</td>
<td>78</td>
</tr>
</tbody>
</table>

Fig. 6. Effect of hydroxylamine and aminoxyactic acid on the concentration of amino acids in brain cortex and liver of rats. Hydroxylamine hydrochloride was injected at level of 75 mg per kg of body weight. Animals were decapitated 90 minutes after injection. Aminoxyactic acid hydrochloride was injected at level of 100 mg per kg of body weight. Animals were decapitated 6 hours after injection. Chromatograms represent ninhydrin-positive compounds in extract of 30 mg equivalents of fresh tissue. Arrows in chromatograms a to e point to spots representing γ-aminobutyric acid. Arrows in chromatograms f to i point to spots identified as β-alanine. The great variability in taurine concentration in the liver of rats is a normal phenomenon and is not the result of drug administration. Origin at right hand bottom of each figure. Horizontal solvent, phenol-water; vertical solvent, butanol-water. The abbreviations used are: NH₂OH, treated with hydroxylamine; AOAA, treated with aminoxyactic acid.
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7. A large number of oximes, hydroxamates, derivatives and analogues of $\gamma$-amino-butyric acid, and glutamic acid, as well as other compounds known to inhibit succinic semialdehyde dehydrogenase in vitro, were injected into rats and failed to elevate levels of $\gamma$-amino-butyric acid in the brains of these animals. The intact amino group of hydroxylamine appears essential for this inhibition.

REFERENCES


Elevation of γ-Aminobutyric Acid in Brain: Selective Inhibition of γ-
Aminobutyric-α-ketoglutaric Acid Transaminase
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