Increases in Tyrosine Hydroxylase Activity after Exposure to Cyclopropane and Fluoxetine

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Prolonged exposure to subanesthetic concentrations of fluoxetine and cyclopropane increased adrenal tyrosine hydroxylase activity in vitro. In addition, cyclopropane increased the activity of tyrosine hydroxylase in the superior cervical ganglion and that of phenylethanolamine-N-methyltransferase in the adrenal. The increase in adrenal tyrosine hydroxylase may be prevented by prior division of the splanchnic nerve fibers which supply the adrenal. The relative increase in enzymic activity in vitro is less than the increase in production of radioactive catecholamines from injected H-tyrosine by the adrenal in vivo. Exposure of rats to 10 per cent cyclopropane for 24 hours produced a significant increase in PaCO₂, but it is doubtful that the changes in enzymic activity observed after cyclopropane are the result of hypercarbia. (Key words: Cyclopropane; Sympathoadrenal activity; Fluoxetine; Hypercarbia; Hypoxia; Tyrosine hydroxylase; Phenylethanolamine-N-methyltransferase; Biosynthesis of neurotransmitters.)

Soon after cyclopropane was first used as a general anesthetic, it was suggested that its cardiovascular effects resulted from an increase in the activity of the sympathetic nerves and/or the adrenal medulla. More than a decade ago, this impression was supported by data obtained from patients and experimental animals by Price et al. It was recently demonstrated that drugs which produce prolonged reflex increases of activity of the sympathetic nervous system in vivo also increase the activity of tyrosine hydroxylase, the rate-limiting enzyme involved in the synthesis of catecholamines from tyrosine. The purpose of the present investigation was to determine whether cyclopropane and fluoxetine, both of which increase preganglionic nerve activity, also alter the activity of the biosynthetic enzymes. The results indicate that prolonged exposure of rats to low, subanesthetic concentrations of cyclopropane increases tyrosine hydroxylase in the adrenal medulla, and that this increased activity can be prevented by division of the splanchnic nerve before exposure to cyclopropane.

Methods

Male Sprague-Dawley rats weighing 110-140 g were obtained from Hormone Assay Laboratories (Chicago, Illinois) and housed in large cages at least three days before use. In 16 designated rats, fibers of the left splanchnic nerve which supplied the left adrenal were divided under ether anesthesia three days before exposure to cyclopropane. In order to determine arterial blood gas tension, a no. 50 Clay-Adams polyethylene catheter was placed in the femoral artery while the rat was anesthetized with ether and brought out behind the neck. These catheters were placed 20 minutes before exposure to cyclopropane and were filled with heparinized saline solution except when samples were removed. The rats were separated within the exposure chamber so that one rat could not reach the sampling catheter of another. All rats were exposed to anesthetic gases in a 55-liter glass and metal chamber which was flushed at a flow rate of not less than 2 l/min (cyclopropane) or 5 l/min (all other studies). After 90 minutes of gas flow at 2 l/min, the gas...
concentration within the exposure chamber was 95 per cent in the delivery tube. Food and water were not available to control or experimental groups. Each chamber contained 300–400 g of soda lime attached to the roof of the cage so that the rats could not reach it. Concentrations of cyclopropane, fluoroxyne, and carbon dioxide were monitored using a model 700 F&M gas chromatograph; oxygen tensions were checked using a paramagnetic meter. For determination of arterial oxygen and carbon dioxide tensions, an IL model 113 was employed.

Each rat was killed by a blow on the head. Both superior cervical ganglia and the adrenals were removed and placed on cracked ice, weighed, and homogenized in 0.25 M sucrose at 4°C using glass homogenizers. The homogenates were centrifuged for 10 minutes at 27,000 × g with a Sorvall RC-2 Refrigerated Centrifuge. Samples of the supernatant fraction were used for assay of tyrosine hydroxylase activity, and protein. Each assay contained 40.5 μmoles of mercaptoethanol; 40 μmoles of potassium phosphate buffer, pH 6.0; 1.95 to 3.1 μmoles of 3,5-ditriítotyrosine (39–52 Ci/mM) (Amer sham-Searle, Des plains, Illinois); 96.9 μmoles of 6,7-dimethyl-5,6,7,8-tetrahydropteridine·HCl·5 H2O (pteridine) (Calbiochem, Los Angeles, California); 25–100 μl of enzyme solution; and 0.25 M sucrose, to a final volume of 85 or 135 μl. After incubation of the mixture at 37°C for 20 minutes, the reaction was stopped by the addition of 0.8 ml 5 per cent trichloroacetic acid. After the removal of trichloroacetic acid-insoluble material by centrifugation, the supernatant was passed over 4 × 0.8-cm columns of Dowex 50 W-X4 (H+), and the effluent, together with a 1.0 ml water wash, was collected in scintillation vials. After addition of a suitable dioxane-base phosphor solution, radioactivity was determined using a Mark I Nuclear Chicago liquid scintillation spectrometer. The enzymic activity of each supernatant fraction was determined using at least two different enzyme concentrations, and the results in every case indicated that the reaction velocity was first-order with respect to enzyme. Phenylethanolamine-N-methyltransferase enzymic activity (PNMT) was determined using a method described previously, except that phenylethanolamine was employed as the substrate.

Endogenous adrenal catecholamines were determined as epinephrine using previously described methods. To compare the conversion of tyrosine to catecholamines in the heart and adrenals of rats exposed to cyclopropane with conversion in control rats, every rat in both groups was given a single bolus injection of 3,5-ditriítotyrosine (88 or 101 mCi), iv, an hour before removal of the heart, adrenals, and a sample of venous blood. Before injection of the labeled tyrosine, pH was adjusted to 8.6 and the solution was passed through an aluminum oxide column. Endogenous tyrosine was determined by the method of Wong et al., and radioactive tyrosine was isolated by the method of Lewander and Jonsen. Labeled catecholamines formed from tyrosine were determined as described previously. Appropriate standards of 3H-norepinephrine and 3H-epinephrine were used in all experiments, and the reported values have been corrected for recovery and contamination.

All observed differences were analyzed for statistical significance using Student's t test.

Results

INCREASE IN ADRENAL TYROSINE HYDROXYLASE ACTIVITY AFTER EXPOSURE TO CYCLOPROpane

After exposure to subanesthetic cyclopropane concentrations (6–18 per cent) for 24 hours, the adrenal tyrosine hydroxylase activity increased significantly (fig. 1). Higher concentrations of cyclopropane were not examined for effects on enzymic activity in vitro because of the need for assisted ventilation with high concentrations. A concentration of 12 per cent cyclopropane produced the maximum increase in enzymic activity. Exposure for 18 hours, however, did produce a significant increase (128 per cent of control, P < 0.05). For this reason, all further studies were conducted for a minimum of 18 hours.

Since section of the splanchnic nerve prevents drug-induced increases in adrenal tyrosine hydroxylase activity, the effect of prior splanchnic-nerve section on the cyclopropane-initiated increase in enzymic activity was determined. Earlier experiments had demon-
strated that after interruption of the splanchnic nerve adrenal tyrosine hydroxylase decreased, with a \( t_1 \) of 21 days.\(^{20}\) For this reason, rats were examined three days after splanchnic-nerve section under ether anesthesia. As a result of the slight decrease in enzymic activity in the adrenals after operation, enzymic activity was expressed as a per cent of that in analogous tissue of control rats. Figure 2 shows that prior splanchnic-nerve transection did prevent the cyclopropane-initiated increase in enzymic activity in the adrenal.

**Effects of Subanesthetic Cyclopropane Concentrations on Extra-adrenal Tyrosine Hydroxylase Activity and Adrenal Phenylethanolamine-N-Methyltransferase Activity**

Previous studies had indicated that, like that of tyrosine hydroxylase, the activity of phenylethanolamine-N-methyltransferase (PNMT) may increase after prolonged exposure to agents which increase the activity of preganglionic sympathetic nerves.\(^{21}\) As can be seen in table 1, rats exposed to 15 per cent cyclo-
Table 1. Effects of Cyclopropane on Catecholamine Biosynthetic Enzymes*

<table>
<thead>
<tr>
<th>Group</th>
<th>Superior Cervical Ganglia</th>
<th>Adrenal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tyrosine Hydroxylase</td>
<td>Tyrosine Hydroxylase</td>
</tr>
<tr>
<td></td>
<td>(mumoles/mg protein/hour)</td>
<td>(mumoles/pair/hour)</td>
</tr>
<tr>
<td>Control</td>
<td>5.6 ± 0.3</td>
<td>11.9 ± 0.5</td>
</tr>
<tr>
<td>Cyclopropane, 15 per cent</td>
<td>7.3 ± 0.3†</td>
<td>17.8 ± 0.4†</td>
</tr>
<tr>
<td></td>
<td>+30 per cent</td>
<td>+35 per cent</td>
</tr>
</tbody>
</table>

* Rats were exposed to 15 per cent cyclopropane/85 per cent air for 24 hours before they were killed. Each value represents mean ± SE of values from six rats.
† P < 0.05
‡ P < 0.01.

Cyclopropane in air have a significant increase in this adrenal enzyme as well. As with other drugs (reserpine, 6-hydroxydopamine, phenoxybenzamine), the relative change in PNMT was less than that observed for tyrosine hydroxylase. The tyrosine hydroxylase activity of superior cervical ganglia of rats exposed to cyclopropane for 24 hours also increased. Again, this change in enzymatic activity in the sympathetic ganglia was slightly less than that observed for this enzyme in the adrenal medulla.

**Effects of Subanesthetic Tensions of Cyclopropane on Arterial Blood Gases**

Since it is widely accepted that cyclopropane produces respiratory depression, arterial CO₂ and O₂ tensions in rats exposed to concentrations of cyclopropane which initiate an increase in tyrosine hydroxylase activity were determined. Exposure to 10 per cent cyclopropane for 24 hours does produce a significant increase in PaCO₂, but the decrease in PaO₂ (9 torr) was not statistically significant (table 2).

**Effect of Exposure to Hypercarbonic Gas Mixtures on Adrenal Tyrosine Hydroxylase Activity**

Since PaCO₂ does increase during the experimental time used in the cyclopropane experiments, it was felt necessary to explore the effect of an increase in inspired FCO₂ on adrenal tyrosine hydroxylase. Exposure to 10 or 15 per cent carbon dioxide in air for 24 hours significantly increased adrenal tyrosine hydroxylase activity (fig. 3). However, this did not occur with 5 per cent carbon dioxide.

**Effect of Hypoxia on Adrenal Tyrosine Hydroxylase Activity**

Although the changes in PaO₂, in rats exposed to 10 per cent cyclopropane were not significant, the mean value after 24 hours of exposure to cyclopropane tended to be lower than the control value. Rats were exposed to low tensions of oxygen which would permit their survival for 18 hours. As can be seen in table 3, there were no significant changes in adrenal tyrosine hydroxylase activity at FiO₂ (fractional inspired concentration of oxygen) 0.132 or 0.068. Lower inspired oxygen tensions were fatal within an hour of starting to flush exposure chambers with the lower concentrations.

Table 2. Effects of 10 Per Cent Cyclopropane on Arterial PaCO₂ and PaO₂*

<table>
<thead>
<tr>
<th>Exposure Time</th>
<th>Number of Rats</th>
<th>PaCO₂ (torr)</th>
<th>PaO₂ (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>35 ± 2</td>
<td>87 ± 15</td>
</tr>
<tr>
<td>4 hours</td>
<td>6</td>
<td>38 ± 2</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>6 hours</td>
<td>4</td>
<td>38 ± 1</td>
<td>83 ± 1</td>
</tr>
<tr>
<td>24 hours</td>
<td>4</td>
<td>43 ± 2†</td>
<td>78 ± 5</td>
</tr>
</tbody>
</table>

* Catheters were placed in the femoral artery before exposure to cyclopropane, as described in methods. Each value represents mean ± SE.
† P < 0.05.
FIG. 3. Effects of hypercarbia on adrenal tyrosine hydroxylase activity. Groups of six rats were exposed to the indicated per cent carbon dioxide in air mixtures (crosshatched) or air only (open column) for 24 hours before they were killed. Each column represents mean ± SE (brackets) of observed enzymic activity, expressed as per cent of the corresponding control value. * P < 0.05.

COMPARISON OF ADRENAL TYROSINE HYDROXYLASE ACTIVITY IN VITRO WITH CATECHOLAMINE SYNTHESIS

Although the increase in tyrosine hydroxylase activity in vitro in adrenals removed after exposure to cyclopropane was significant, the synthesis of catecholamines in vivo would not necessarily be increased by a proportional amount. For this reason, conversion of $^3$H-tyrosine to $^3$H-catecholamines was determined.

Four hours after exposure to cyclopropane in vivo (table 4), synthesis of catecholamines from tyrosine was significantly increased in the adrenal (+29 per cent), although tyrosine hydroxylase activity in these adrenals in vitro had not yet increased significantly (+9 per cent). No significant increase in conversion of catechols from tyrosine was detected in the heart. Twenty-four hours after exposure to cyclopropane, the relative increase in synthesis of catechols from tyrosine in the adrenal in vivo was again much greater than the change in adrenal tyrosine hydroxylase activity in vitro. At 24 hours, the content of labeled catechols in the hearts was not significantly greater.

EFFECTS OF OTHER ANESTHETIC AGENTS ON ADRENAL TYROSINE HYDROXYLASE ACTIVITY

Exposure of rats of subanesthetic doses of halothane (0.5 per cent) and nitrous oxide (75 per cent) for 24 hours did not provoke any significant change in adrenal tyrosine hydroxylase activity. However, exposure of fluroxene produced a significant elevation of adrenal tyrosine hydroxylase activity (table 5). There was no significant change in tyrosine hydroxylase activity in the superior cervical ganglia or in adrenal PNMT activity in rats given fluroxene. Catechol synthesis from labeled tyrosine precursor in vivo was not examined in rats exposed to fluroxene.

Discussion

The results indicate that exposure of rats to subanesthetic concentrations of cyclopropane

<table>
<thead>
<tr>
<th>Group</th>
<th>Ambient Oxygen Tension (torr)</th>
<th>Adrenal Tyrosine Hydroxylase (mumoles/pair/hour)</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6 rats)</td>
<td>143</td>
<td>3.94 ± 0.22</td>
<td>+8 per cent</td>
</tr>
<tr>
<td>Hypoxic (7 rats)</td>
<td>90</td>
<td>4.27 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>Control (6 rats)</td>
<td>142</td>
<td>4.98 ± 0.59</td>
<td>+10 per cent</td>
</tr>
<tr>
<td>Hypoxic (8 rats)</td>
<td>50</td>
<td>5.47 ± 0.51</td>
<td></td>
</tr>
</tbody>
</table>

* Rats were exposed to the designated environment for 18 hours before they were killed. Each value represents mean ± SE of the number of determinations (one determination per rat).
or fluroxene increases the activity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, in vivo. Both agents are known to increase the electrical activity of sympathetic preganglionic neurons at anesthetic concentrations in the cat, possibly as a result of suppression of the baroreflex. No similar studies have been done in the rat. Since division of the fibers of the splanchnic nerve which supplies the left adrenal prevented the increase in adrenal tyrosine hydroxylase provoked by exposure to cyclopropane, at least intact innervation is necessary, and indeed the impulses initiating the increase in enzyme activity may arrive via the splanchnic nerve. Although hypophysectomy also decreases adrenal tyrosine hydroxylase activity secondary to a decrease in adrenal cortical activity, neither stimulation of the rat adrenal cortex by large doses of ACTH (20 units/kg) nor administration of dexamethasone (1 mg/kg) increased adrenal tyrosine hydroxylase activity. Therefore, cyclopropane and fluroxene appear to resemble other drugs (reserpine, 6-hydroxydopamine, phenoxybenzamine) which increase sympathetic nerve activity and also increase the activity of adrenal tyrosine hydroxylase.

Recent investigations of the uptake, release, and biosynthesis of norepinephrine in animals anesthetized with cyclopropane failed to reveal any alteration in the dynam-
ics of norepinephrine in peripheral adrenergic nerves, including the rat heart. The present results, using a single intravenous administration of labeled tyrosine rather than a constant infusion, confirm that cyclopropane does not alter either the endogenous norepinephrine concentration or the amount of labeled catecholamine derived from labeled tyrosine in the rat heart. In contrast to the findings in the heart, however, a marked increase in newly synthesized labeled catecholamines was found in the adrenal medulla. While a single injection of the precursor, $^3$H-tyrosine, does not permit calculation of the rate of synthesis, changes in rate of synthesis can be estimated. This increased conversion was greater than the relative increase in tyrosine hydroxylase activity in vitro. Thus, the relative changes in synthesis in vivo relative to activity in vitro in the present study resemble recent results with phenoxybenzamine. The rapid minute-to-minute adjustments in tyrosine hydroxylase activity in vivo are accomplished not by changing the amount of the rate-limiting enzyme tyrosine hydroxylase, but rather by altering end-product inhibition of the enzyme by norepinephrine and epinephrine. The increase in the activity of tyrosine hydroxylase in vivo is seen only after prolonged increased activity in preganglionic or splanchnic nerves.

The observed increase in PNMT activity in the adrenal medulla resembles that of rats given 6-hydroxydopamine, and similarly indicates that alternations in the amount of this enzyme, which converts norepinephrine to epinephrine, may be accomplished by similar changes in the other intermediate enzymes in catecholamine synthesis.

The increase in tyrosine hydroxylase activity in vitro in the cell bodies of adrenergic neurons in the superior cervical ganglion is not inconsistent with the absence of any increase of synthesis in vivo in the adrenergic nerve terminals of the heart. Changes in tyrosine hydroxylase activity in nerve terminals occur only several days after alterations in cell body enzyme are fully developed.

It is possible that the present findings are related to the increase in circulating catecholamines produced by cyclopropane. Price et al. did not find any consistent change in plasma epinephrine concentration in patients given cyclopropane. Although it could be argued that the adrenals may be releasing predominantly norepinephrine, adrenalectomized patients still manifested an increase in circulating norepinephrine. These data imply that the adrenal does not contribute to the increase in circulating norepinephrine. It is possible that, like the dog, the rat may respond to cyclopropane by stimulation of the adrenal medulla instead of the sympathetic nerves, opposite to the response in man. It should be emphasized also that results obtained with subanesthetic concentrations may not be the same as those which would occur with anesthetic concentrations.

It has been proposed that, like cyclopropane, fluroxene may stimulate the sympathetic nervous system. This has been supported by the demonstration of an increase in the activity of preganglionic sympathetic nerves of cats and the finding of increases in circulating norepinephrine and epinephrine in human beings during fluroxene anesthesia. The present findings of an increase in adrenal tyrosine hydroxylase activity after prolonged exposure to subanesthetic concentrations of fluroxene may again reflect the consequences of prolonged elevation of splanchnic-nerve activity.

Although in the present study only the effects of subanesthetic concentrations are reported, the prolonged exposure necessary for demonstration of the increase in biosynthetic enzymic activity suggested that ventilatory function should be assessed. Since mild carbon dioxide retention was demonstrated during cyclopropane administration, the effect of carbon dioxide on adrenal tyrosine hydroxylase activity was determined. Fenn and Asano previously proposed that hypercapnia increases sympathoadrenal activity in the cat, which was confirmed by the demonstration of an increase in circulating catecholamines. Nahus and Steinsland have recently demonstrated increased conversion of $^3$H-tyrosine to labeled catecholamines in the heart and adrenals of rats during exposure to 20 per cent CO$_2$. This increased enzymic activity in vivo probably reflects an increase in effenter sympathoadrenal activity. Such an increase in activity maintained for many hours would then be the likely explanation of the increased activity of
tyrosine hydroxylase in vitro observed in the present study in rats exposed to 10 or 15 per cent carbon dioxide. It seems unlikely that the increase in enzymic activity produced by cyclopropane is the result of retention of carbon dioxide or hypoxia.

The activity of the drug-metabolizing enzyme system in the liver can be increased or decreased by prior administration of other drugs and by environmental and pathologic factors. These changes in activity are of major importance in explaining the clinical effects of certain drug combinations and abnormal drug responses as a consequence of hepatic or systemic disease. Like the micosomal enzymes of the liver, tyrosine hydroxylase and probably other enzymes involved in the synthesis of adrenergic neurotransmitters, can be induced by drugs, physical stress and psychosocial stress and pathologic conditions such as hypertension and chemical myocardial. These changes are not limited to the peripheral sympathoadrenal system, but can occur in the central noradrenergic nerves as well. It is unreasonable to assume that drug-induced or pathologically-induced changes in this enzyme, which regulates catecholamine synthesis, may be present in patients about to undergo anesthesia. Such changes may alter the basal rate of synthesis of catecholamines and/or alter the ability of the patients to respond to the stress of surgery and anesthesia. In addition, the present results suggest the possibility that prior exposure to the anesthetic agent itself may alter the subsequent capacity for neurotransmitter synthesis.

The author gratefully acknowledges the expert technical assistance of Miss Elena Molina and Mrs. Alice Foster.

References

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Measurements of respiratory function and arterial blood gases were carried out at various CPAP levels. Arterial oxygenation improved in all infants once CPAP was instituted. This allowed for an average decrease in the inspired oxygen tension of 37.5 per cent within 12 hours after initiation of therapy. P CO2 was not appreciably altered by CPAP, but tidal and minutes volumes of respiration decreased. No consistent changes in heart rate or blood pressure occurred. The increase in intrathoracic pressure was approximately 20 per cent of the applied airway pressure. A patent ductus arteriosus became clinically apparent in nine of the 20 infants.

CPAP represents an improvement in treatment of infants with the respiratory distress syndrome. These initial studies achieved a significantly greater survival rate than would be expected with other methods of support used to date. Complications secondary to the use of CPAP are not forbidding, although further studies are necessary to prove the validity of this point. The improvement in oxygenation without alteration in P CO2 indicates that CPAP improves the relationship between ventilation and perfusion without significantly increasing alveolar ventilation. (Gregory, G. A., and others: Treatment of the Idiopathic Respiratory Distress Syndrome with Continuous Positive Airway Pressure, New Eng. J. Med. 284: 1333 (June 17) 1971.)