Effect of Aminophylline on Urinary Excretion of Epinephrine and Norepinephrine in Man

By Nuzhet O. Atuk, M.D., M. Cary Blaydes, M.D., Frederic B. Westervelt, Jr., M.D., and J. Edwin Wood, Jr., M.D.

SUMMARY

The excretion of catecholamines and the changes in plasma concentration of free fatty acids during aminophylline administration were explored. The relationships between cardiac arrhythmia, cardiac rate, and change in blood pressure under the conditions of these experiments were defined. Eighteen experiments were performed on eight volunteers. Blood pressures and heart rates before and during aminophylline infusion were recorded at frequent intervals, and urine and blood were collected during the control and infusion periods and in some subjects after the infusion. Loading with ethanol, glucose, or placebo before administration of aminophylline was used.

These studies demonstrated that intravenous infusion of aminophylline increases the urinary excretion of epinephrine and norepinephrine in man, the rate of excretion of epinephrine being greater than that of norepinephrine. This increase was accompanied by an increase in the concentration of free fatty acids in the plasma.

ADDITIONAL INDEXING WORDS:
Catecholamines  Diuresis  Ethanol  Theophylline
Blood pressure  Heart rate  Blood lipids  Arrhythmia
Glucose  Autonomic nervous system

During experiments originally designed to study free water clearance using ethanol and aminophylline, changes in cardiac rhythmicity and increased vasomotor activity were observed. This suggested an additional pharmacological effect of one or both of these drugs mediated through the sympathetic nervous system. Anton has documented such an effect by ethanol. Cardiac arrhythmias, change in blood pressure, tremulousness, and sweating as well as sudden death during aminophylline administration have been linked to catecholamines only by inference. The present study was designed to explore further this relationship.

Methods

Eighteen experiments were performed on eight male volunteers ranging in age from 23 to 42 years. On the morning of the experiment, the subjects ate a light breakfast consisting of cereal and milk. Neither coffee nor cigarettes were allowed prior to or during the experiment. An indwelling forearm intravenous needle was kept patent by a slow (0.75 ml/min) infusion of 5% glucose in water throughout the experiment. After the initial urine was discarded, the subjects assumed a supine position and were given 500 to 1,000 ml of water orally, maintaining a positive water balance for the duration of the experiment. The blood pressures and heart rates before and during aminophylline infusion were recorded for 10 of the 12 studies at frequent intervals (2 to 5 min). The subjects voided at 15 to 30 minute intervals during the control (−60 to 0 min) and infusion (0 to 75 min) periods. In several subjects postinfusion (75 to 135 min)
urines were collected in the same manner. When oral theophylline was employed, it was given at the onset of the infusion period, and the urine collections were made similarly. Anxiety and discomfort were minimized as much as possible.

In six experiments, the subjects were primed with ethanol (45 ml in the form of 3 ounces of 100 proof whiskey) at −60 min. Aminophylline, 500 mg/100 ml of 5% dextrose in water, was then administered at a constant rate during the infusion period. In six additional studies the subjects received 50 g of glucose orally at −60 min instead of ethanol, followed by aminophylline in the usual fashion. Placebo studies were carried out in six subjects who received, without their knowledge, isotonic saline rather than aminophylline during the infusion period.

Urine was analyzed for epinephrine (E) and norepinephrine (NE) by the von Euler method. In all instances, 50-ml aliquots of urine were used. After the urine was boiled and filtered, the disodium salt of ethylene diamine tetra-acetic acid (EDTA) was added, pH was adjusted to 8.5 with 0.5 N NaOH, before it was passed through an aluminum oxide column. E and NE were then eluted with H2SO4 and neutralized with NaHCO3. Oxidation was carried out with potassium ferricyanide. The catecholamines were then transformed to stable lutines with NaOH and ascorbic acid. The fluorescence of the solutions was measured against a standard using a Coleman model 12C photofluorometer. The values for E and NE were not corrected for analytic losses. The recoveries averaged 73.9% (sd ± 10.7) and 72.0% (sd ± 9.67), respectively, for E and NE when 1, 1.5, 2, and 40 µg were added to the urine samples.

Samples of heparinized blood were obtained frequently from a separate vein during the control, infusion, and postinfusion periods. The samples were promptly immersed in ice water and analyzed for free fatty acid (FFA) by the modified method of Dole. Data were analyzed statistically by the t-test using the difference method for correlated scores, since each subject served as his own control. Results are expressed as mean ±se (standard error) throughout.

Results

Blood Pressure and Heart Rate Response to Aminophylline

The systolic and diastolic blood pressures and heart rates before and during aminophylline infusion were recorded for 10 of the 12 studies at frequent intervals. The mean systolic blood pressure increase in four subjects was 11 mm Hg (range +3 to +16); in six subjects it remained unchanged. The mean increase in diastolic pressure in eight subjects, was 7 mm Hg (range +3 to +14); in one it fell by 4 mm Hg and in another remained unchanged.

The heart rate in one subject increased (+5 beats/min), in another decreased (−10 beats/min), and in the remaining showed no significant change. In two subjects, premature ventricular contractions occurred during aminophylline infusion and disappeared after the termination of the infusion. Two subjects developed tachycardia (120 and 130 beats/min) on standing up after the termination of the experiment. The Valsalva maneuver in one of these subjects (N.A.) slowed the heart rate to the normal range abruptly, with a brief period of nodal rhythm.

Urinary Cathecholamine Response

In the six ethanol-primed subjects, E excretion was increased during aminophylline infusion; the mean control excretion rate was 1.41 ± 0.67 µg/hr, and that during aminophylline infusion was 4.14 ± 1.65 µg/hr. The mean difference was not statistically significant. The excretion of NE was also increased in these subjects, the mean control excretion rate being 1.44 ± 0.49 µg/hr and during the infusion period being 3.13 ± 0.70 µg/hr (P < 0.01).

In the glucose-primed subjects, the urinary excretion of E was increased in all instances, and that of NE, in all but one, during the aminophylline infusion. The mean control value for E was 1.58 ± 0.44 µg/hr and for NE was 1.44 ± 0.38 µg/hr. During the infusion the mean value for E was increased to 4.82 ± 0.50 µg/hr (P < 0.01) and for NE to 3.13 ± 0.69 µg/hr (P < 0.05).

Placebo saline infusion did not alter E and NE excretion rate in six subjects.

The magnitude of the urinary catecholamine response varied considerably from individual to individual. However, a significant increase in the urinary excretion of E and NE correlated well with a significant rise in the diastolic blood pressure in eight of the 10 subjects. In one subject, the increased excretion of both catecholamines was not accompanied by any change in blood pressure. In another
Table 1
Response of Urinary Epinephrine and Norepinephrine to Intravenous Infusion of Aminophylline in Ethanol and Glucose-Primed Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Ethanol and aminophylline</th>
<th>Glucose and aminophylline</th>
<th>Placebo saline infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urinary collection periods</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epinephrine excretion (µg/hr)</td>
<td>Norepinephrine excretion (µg/hr)</td>
<td>Creatinine clearance (mL/min)</td>
</tr>
<tr>
<td></td>
<td>Control*</td>
<td>Infusion*</td>
<td>Control*</td>
</tr>
<tr>
<td>J.L.</td>
<td>4.50</td>
<td>11.66</td>
<td>3.41</td>
</tr>
<tr>
<td>N.A.</td>
<td>0.75</td>
<td>2.00</td>
<td>1.81</td>
</tr>
<tr>
<td>F.W.</td>
<td>0.50</td>
<td>0.56</td>
<td>0.46</td>
</tr>
<tr>
<td>W.B.</td>
<td>2.04</td>
<td>3.13</td>
<td>0.00</td>
</tr>
<tr>
<td>C.B.</td>
<td>0.16</td>
<td>1.89</td>
<td>1.12</td>
</tr>
<tr>
<td>N.A.</td>
<td>0.56</td>
<td>5.60</td>
<td>1.84</td>
</tr>
<tr>
<td>Mean</td>
<td>1.41</td>
<td>4.14</td>
<td>1.44</td>
</tr>
<tr>
<td>se ±</td>
<td>0.67</td>
<td>1.65</td>
<td>0.49</td>
</tr>
<tr>
<td>Significance† ns‡</td>
<td>P &lt; 0.01</td>
<td>ns‡</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

J.L. 2.65 4.58 2.66 2.94 192 165
J.B. 0.71 5.72 0.12 0.91 148 167
F.W. 0.87 2.66 1.54 1.47 128 124
C.A. 0.48 5.58 0.54 3.64 146 141
C.B. 3.07 6.00 1.84 4.84 125 109
M.O. 1.69 4.40 1.94 5.04 133 131
Mean 1.58 4.82 1.44 3.13 145 139
se ± 0.44 0.50 0.38 0.69 3.8 3.5

Placebo saline infusion

<table>
<thead>
<tr>
<th>Subjects</th>
<th>urinary collection periods</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epinephrine excretion (µg/hr)</td>
<td>Norepinephrine excretion (µg/hr)</td>
<td>Creatinine clearance (mL/min)</td>
</tr>
<tr>
<td></td>
<td>Control*</td>
<td>Infusion*</td>
<td>Control*</td>
</tr>
<tr>
<td>J.L.</td>
<td>2.64</td>
<td>2.61</td>
<td>1.93</td>
</tr>
<tr>
<td>J.B.</td>
<td>3.36</td>
<td>5.70</td>
<td>4.20</td>
</tr>
<tr>
<td>F.W.</td>
<td>3.90</td>
<td>2.66</td>
<td>0.90</td>
</tr>
<tr>
<td>C.A.</td>
<td>1.58</td>
<td>1.38</td>
<td>2.50</td>
</tr>
<tr>
<td>C.B.</td>
<td>1.57</td>
<td>0.93</td>
<td>2.80</td>
</tr>
<tr>
<td>N.A.</td>
<td>4.15</td>
<td>1.29</td>
<td>3.08</td>
</tr>
<tr>
<td>Mean</td>
<td>2.66</td>
<td>2.42</td>
<td>2.56</td>
</tr>
<tr>
<td>se ±</td>
<td>0.45</td>
<td>0.71</td>
<td>0.45</td>
</tr>
<tr>
<td>Significance† ns‡</td>
<td>ns‡</td>
<td>ns‡</td>
<td>ns‡</td>
</tr>
</tbody>
</table>

*Urinary catecholamine excretion values represent the entire control 1-hour period and infusion period 1 hour and 15 minutes.
†Significance of changes of epinephrine, norepinephrine, and creatinine clearance during intravenous aminophylline infusion.
‡ns = not significant.

subject, a decrease in excretion of norepinephrine was coincident with a minimal decrease in diastolic blood pressure.

Plasma FFA Response to Aminophylline

In order to examine catecholamine activity indirectly, in seven subjects FFA levels were determined at frequent intervals (table 2).

An example of the FFA changes in a typical ethanol-primed subject (J.L.) is shown in figure 1. A prompt rise occurred in plasma FFA during the aminophylline infusion. In this subject, as in the other two subjects, FFA levels increased \( P < 0.01 \); the peak increments ranged from +891 to +1,250 µEq/L. The time interval for the occurrence of the peak varied from 10 to 45 minutes (table 2). The rise in FFA was somewhat modified in the four glucose-primed subjects who were studied, as exemplified by subject C.A. (fig. 1).

Placebo saline infusion did not alter plasma FFA concentrations in six subjects.

FFA response in the two test groups is com-

Circulation, Volume XXXV, April 1967
Table 2

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control Infusion period (min)</th>
<th>Plasma FFA concentrations (μEq/L)</th>
<th>Postinfusion 90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 to 0</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Ethanol and aminophylline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.L.</td>
<td>408</td>
<td>1299</td>
<td>886</td>
</tr>
<tr>
<td>N.A.</td>
<td>771</td>
<td>968</td>
<td>1224</td>
</tr>
<tr>
<td>F.W.</td>
<td>297</td>
<td>764</td>
<td>762</td>
</tr>
<tr>
<td>Mean</td>
<td>492</td>
<td>1010</td>
<td>957</td>
</tr>
<tr>
<td>T.E.</td>
<td>143</td>
<td>155</td>
<td>138</td>
</tr>
<tr>
<td>Significance*</td>
<td>ns† P &lt; 0.01</td>
<td>ns</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Glucose and aminophylline

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control Infusion period (min)</th>
<th>Plasma FFA concentrations (μEq/L)</th>
<th>Postinfusion 90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.L.</td>
<td>223</td>
<td>248</td>
<td>323</td>
</tr>
<tr>
<td>J.B.</td>
<td>303</td>
<td>338</td>
<td>328</td>
</tr>
<tr>
<td>F.W.</td>
<td>303</td>
<td>303</td>
<td>288</td>
</tr>
<tr>
<td>C.A.</td>
<td>378</td>
<td>378</td>
<td>378</td>
</tr>
<tr>
<td>Mean</td>
<td>301</td>
<td>316</td>
<td>311</td>
</tr>
<tr>
<td>T.E.</td>
<td>31</td>
<td>27</td>
<td>44</td>
</tr>
<tr>
<td>Significance*</td>
<td>ns† P &lt; 0.01</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Placebo saline infusion

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control Infusion period (min)</th>
<th>Plasma FFA concentrations (μEq/L)</th>
<th>Postinfusion 90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.L.</td>
<td>350</td>
<td>296</td>
<td>279</td>
</tr>
<tr>
<td>J.B.</td>
<td>443</td>
<td>615</td>
<td>638</td>
</tr>
<tr>
<td>F.W.</td>
<td>399</td>
<td>296</td>
<td>301</td>
</tr>
<tr>
<td>C.A.</td>
<td>602</td>
<td>541</td>
<td>535</td>
</tr>
<tr>
<td>C.B.</td>
<td>323</td>
<td>142</td>
<td>125</td>
</tr>
<tr>
<td>Mean</td>
<td>217</td>
<td>274</td>
<td>228</td>
</tr>
<tr>
<td>T.E.</td>
<td>389</td>
<td>360</td>
<td>351</td>
</tr>
<tr>
<td>Significance*</td>
<td>ns† P &lt; 0.01</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Significance of peak Δ FFA in ethanol and glucose-primed subjects showed differences with values of (P < 0.01) and (P < 0.05).
†ns = not significant.

pared with that of the placebo group. The ratio of plasma FFA levels during infusion time to the control levels is depicted in figure 2. The deviation of the ratio from unity as well as statistical analysis of the actual data (table 2) indicates that the peak FFA concentrations differed significantly from the control values. This is the response one might expect when E or NE is administered. As anticipated, this response is to some extent modified in glucose-primed subjects.

The possibility that aminophylline and theophylline interfere with the chemical analysis for the catecholamines could not be excluded. However, it has been reported that only very small amounts are excreted unchanged when theophylline and caffeine are given to man. Furthermore, fluorescence of xanthines is extremely low. In this laboratory, aminophylline and theophylline were added in different concentrations to urine and no demonstrable differences were found in the concentrations of catecholamines (table 3).

Discussion

These studies demonstrate that intravenous aminophylline increases the urinary excretion of E and NE in man, with the E excretion rate greater than that of NE. This increase in catecholamine excretion could occur through several mechanisms.

This increase could be due to diuresis itself. However, when excretion rates of E and NE were determined in the urines which were collected in short intervals following the aminophylline infusion in two subjects,
The urinary catecholamine and plasma FFA response to aminophylline given intravenously to an ethanol-primed subject (J.L.) and to a glucose-primed subject (C.A.), with placebo experiments as indicated.

This rate was highest in the first collection period (15 to 30 min) (see subject C.A., fig. 3). The results further indicate that the increment in E and NE excretion rates was not directly related to that of urine flow. This finding was supported by the observation of von Euler and Anton that the amount of catecholamines excreted per unit of time is largely independent of urinary flow rate. These observations, together with the response in plasma FFA (see below) suggest that the urinary excretion of catecholamines is dependent upon the rate of release of catecholamines into plasma rather than upon the urinary flow rate.

Recently, aminophylline diuresis has been reported to be abolished in reserpine fed hens and with restoration of the diuretic effect later by administering the free catecholamine precursor, dopamine. This evidence supports our view that catecholamines play an intermediary role in this diuretic effect.

The possibility that this increased catecholamine excretion could be due to the increased glomerular filtration rate is not supported by our data.

The role of the system which actively transports E from blood to urine across the tubule cell is also to be considered. It is known that E is transported by the organic acid pathway. It has been reported also that not more
than 10% of the aminophylline is excreted intact; the remainder is broken down and excreted as methylxanthines or as methyluric acid. Such substances would not reasonably be expected to augment E excretion simply by interfering with the active transport system.\textsuperscript{16} Ethanol has been shown to cause an increase in urinary excretion of E and NE in man\textsuperscript{1,17} and in the cat.\textsuperscript{18} Acute sublethal alcohol intoxication of intact unanesthetized dogs is accompanied by a marked increase in urinary excretion of E and NE.\textsuperscript{19} Since this effect was present to some extent in the ethanol-primed subjects, catecholamine excretion was compared in ethanol-primed subjects with that of subjects who were given aminophylline alone. The comparison of the excretion rates between the two groups showed no significant difference.

E and NE have been found to promote fat mobilization, as demonstrated by experiments both in vitro\textsuperscript{20,21} and in vivo.\textsuperscript{8,22} Bogdonoff and associates\textsuperscript{23} have demonstrated that, among the catecholines and other vasoactive compounds studied, certain chemical

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Concentrations of the & Norepinephrine & Epinephrine \\

drug tested in urine\textsuperscript{*} & (\mu g hr) & (\mu g hr) \\
\hline
Control & 0.69 & 0.48 \\
Urine 1 & 0.45 & 0.12 \\
& (10 mg/25 ml & \\
& theophylline) & \\
Urine 2 & 0.85 & 0.53 \\
& (1 mg/25 ml & \\
& theophylline) & \\
Urine 3 & 0.93 & 0.71 \\
& (1 mg/25 ml & \\
& aminophylline) & \\
Urine 4 & 0.60 & 0.41 \\
& (10 mg/25 ml & \\
& aminophylline) & \\
\hline
\end{tabular}
\caption{Analysis of Urinary Catecholamines When Aminophylline and Theophylline Are Added}
\end{table}

\textsuperscript{*}Concentrations are assumed to be comparable to those present in the extracellular fluid, since the drug is soluble in water.
AMINOPHYLLINE ON URINARY CATECHOLAMINES

Figure 4

The urinary catecholamine and plasma FFA response to ethanol alone.

structural configurations are necessary for a significant lipolytic effect. E and NE, along with the synthetic compound isoproterenol, are the only members of this group that have this property. Klein and associates8 have shown also that continued infusion of NE at the rates of 4 to 5 μg/min causes the peak of response in FFA in approximately 30 minutes. In the light of these observations, our demonstrated increases in the plasma FFA levels and the peak responses suggest the release of large quantities of catecholamines following aminophylline infusion.

The question arises whether the FFA increase is directly related to the increased release of E and NE as produced by the ethanol or indirectly related to aminophylline. In the rat, the observations of Brodie and Maickel24 and Mallov25 indicated that the increase in plasma FFA occurred when intoxicating doses of alcohol were given. In these experiments blood levels of alcohol increased above 200 mg/100 ml. However, Freinkel and associates26 have found that ethanol infusion induces an early decline in FFA levels, dissociated from any effect on blood glucose and that this decline persists for as long as 2 hours despite plasma ethanol levels as high as 100 mg/100 ml. In the present study plasma FFA levels were followed in two subjects following ethanol ingestion alone. A typical FFA curve is depicted in figure 4. In this subject as in the other one plasma FFA levels decreased after ethanol alone.

The modified FFA response to aminophylline in our glucose-primed subjects does not vitiate the possibility of increased catecholamine elaboration, for it has been recently shown27 that prior or concomitant administration of glucose modified the rise of FFA levels after administration of epinephrine.

The possible role of emotional stress in the release of catecholamines and promotion of fat mobilization has been considered. Anxiety and tension may be accompanied by an increase in the excretion of catecholamines28–30 as shown in experiments conducted in man31–33 and in animals.22 Stimulation of the autonomic nervous system has been found to influence the plasma FFA level also. Although it is not possible to separate entirely psychophysiological and pharmacological responses, extreme care was taken to reduce anxiety and discomfort in our subjects during these experiments. Placebo infusion was not associated with a significant increase in urinary excretion of catecholamines or with a rise in FFA levels. It was hoped that anxiety and stress would be further minimized by administration of theophylline by mouth. The results, shown in figure 5, indicate that the drug has the same effect on urinary catecholamines and plasma FFA levels. This approach has the additional advantage of using xanthine without ethylene diamine.

At present, the rise of urinary excretion rates of NE and E is considered to be a good measure of sympathetic and adrenomedullary activity.29 Admittedly, rebinding of free NE and its rapid transformation into its metabolites after its release may influence its appearance in the urine. But increased secretion from the adrenal medulla is the most likely explanation for the increased urinary excretion of E.

In view of the observations, our demonstrated increase in urinary excretion of both
E and NE, with relatively high values for the former, suggests that aminophylline stimulates the adrenal gland to release these catecholamines. Whether or not aminophylline similarly causes release of catecholamines from the sympathetic system cannot be determined by this study.

**Conclusions**

1. Urinary epinephrine and norepinephrine excretions are found to be significantly increased following intravenous infusion of aminophylline in therapeutic doses.

2. The increase in catecholamine excretion is accompanied by an increase in plasma FFA concentration, suggesting an increased rate of release of catecholamines.

3. It is suggested that aminophylline stimulates the adrenal medulla and possibly chromaffin tissue situated outside the adrenal gland to release catecholamines.

**Acknowledgment**

We would like to thank Dr. K. R. Crispell for his encouragement in this study, Dr. Guy Hollifield for analyzing the plasma free fatty acids, Dr. A. D. Hart for his careful review of the manuscript, Dr. Preston B. Lowrance for his assistance during various phases of this study, and Mrs. Peter Wiernik, Mrs. Robert A. Harris, Jr., and Mr. Joseph G. Vann for their technical assistance.

**References**


_Circulation, Volume XXXV, April 1967_
Effect of Aminophylline on Urinary Excretion of Epinephrine and Norepinephrine in Man

NUZHET O. ATUK, M. CARY BLAYDES, FREDERIC B. WESTERVELT, JR. and J. EDWIN WOOD, JR.

Circulation. 1967;35:745-753
doi: 10.1161/01.CIR.35.4.745

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/35/4/745