Effect of Intravenous Aminophylline on Plasma Levels of Catecholamines and Related Cardiovascular and Metabolic Responses in Man

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SUMMARY Theophylline is thought to act by inhibiting the activity of phosphodiesterase, with a resultant increase in intracellular cyclic AMP. However, this concept is largely based on in vitro studies using concentrations of theophylline which greatly exceed therapeutic plasma concentrations. To investigate the relationship of the cardiovascular and metabolic effects of theophylline to activation of the sympathetic nervous system, i.v. aminophylline was administered to six healthy males under basal conditions. Each subject received four infusions. Mean theophylline concentrations (± SEM) of 4.5 ± 0.2, 10.0 ± 0.5, 14.0 ± 0.5 and 20.0 ± 1.2 µg/ml were achieved. Plasma epinephrine increased 262% (from 29 ± 4 to 105 ± 14 pg/ml, p < 0.01) and plasma norepinephrine increased 64% (from 190 ± 18 to 312 ± 51 pg/ml, p < 0.05) during the high-dose infusion. The increases in circulating catecholamines were dose-related (p < 0.001 by analysis of variance). Dose-related increases in heart rate, systolic blood pressure, plasma glucose, free fatty acids and insulin were also observed (p < 0.001 by analysis of variance). Although the duration of total electromechanical systole (QS2) and left ventricular ejection time adjusted for heart rate fell during the aminophylline infusions, this positive isotropic response was not influenced by dose, except possibly the high dose. Echocardiographic ejection fraction was not changed by the aminophylline infusions. We conclude that the acute cardiovascular and metabolic effects of theophylline may be mediated in part by stimulation of the sympathetic nervous system.

AMINOPHYLLINE (theophylline ethylenediamine) is a methylxanthine derivative that is widely used in the treatment of cardiovascular and respiratory disease. Although ethylenediamine has been implicated in allergic reactions to aminophylline, it is otherwise thought to be therapeutically inactive. Theophylline and other methylxanthines are known to exert a variety of important physiologic and biochemical effects.1 Although the dose-response relationship between plasma concentrations of theophylline and improvement of pulmonary function during bronchospasm is well established,2,3 a similar relationship for its cardiovascular and metabolic effects has only recently been examined in human studies.4-6 Despite the evidence that the acute administration of methylxanthine derivatives stimulates the release of epinephrine and norepinephrine from the sympathoadrenal system in man,7,8 the extent to which this response may be dose- or concentration-dependent has only been studied in an in vitro system, the isolated perfused adrenal gland.9,10 Epinephrine and norepinephrine are known to have chronotropic and inotropic effects on the heart, which, together with vasoconstrictor effects, lead to an increase in systolic blood pressure. They also elevate plasma concentrations of glucose and free fatty acids (FFAs), but inhibit insulin secretion. Theophylline has cardiovascular effects similar in many respects to those of the catecholamines.4,11-13 Although theophylline increases plasma FFAs,5,7,14-15 reports of its effect on plasma glucose and insulin are conflicting.5,14,15 To investigate the relationship of the cardiovascular and metabolic effects of theophylline to activation of the sympathetic nervous system, we conducted a dose-response study in which i.v. aminophylline was administered to healthy young men. The increased levels of circulating catecholamines support the hypothesis that some of the acute pharmacologic effects of theophylline may be a result of its effects on the sympathetic nervous system.

Methods

Six healthy young male subjects, ages 18–25 years and weighing 67–84 kg (102–127% of ideal body weight*), participated in a series of four 120-minute aminophylline infusions, which were conducted between 8:00 a.m. and 12:30 p.m. in the Cardiac Testing Laboratory of the Veterans Administration (VA) Medical Center, Boise, Idaho. Subjects had a normal history, physical examination, and screening laboratory evaluation, including a normal ECG. None was taking medications. All studies were performed after an overnight fast, including abstinence from tobacco for at least 8 hours. Subjects also abstained from dietary methylxanthines and alcohol for 48 hours before study. Each subject initially underwent an infusion designed to achieve a mean plasma theophylline concentration of 25 µg/ml. Theophylline was then infused at a rate calculated to achieve plasma concentrations of 100, 200, and 300 µg/ml. Informed consent was obtained from each subject prior to the start of the study.

*The level of weight range according to frame size for male subjects from the 1959 Metropolitan Life Insurance Company table for desirable weight was used.
centration of 10 µg/ml. This was followed by three infusions calculated to achieve plasma theophylline concentrations of 5, 15 and 20 µg/ml. Infusions were performed 2–4 weeks apart and were not randomized so as to permit adjustments in infusion rates necessary to minimize excessive deviations from goal plasma levels and adverse effects. Despite this precaution, one subject developed nausea during his final (high-dose) study and was excluded from the analysis of data for that dose level. The protocol for this study was approved by the Human Studies Review Committees of the VA Medical Center and the University of Washington. All subjects gave informed consent.

After voiding, each subject assumed the supine position and remained supine throughout the study. A 19-gauge butterfly needle was inserted into a large vein in the right forearm for blood sampling without tourniquet pressure and was maintained patent with a slow-drip infusion of 0.9% saline solution. A 21-gauge butterfly needle was inserted into a vein of the left forearm for drug administration and was likewise kept patent with a drip infusion of 0.9% saline solution.

An initial blood sample was obtained and transferred to a chilled, heparinized tube on ice to prepare standards for assay of plasma theophylline and for assay of plasma binding of theophylline. At −30 minutes, a slow i.v. infusion of 0.9% saline was begun through the butterfly needle in the right forearm from one of two 200-ml syringes on a model 2205 Harvard pump equipped with a model 552 pump speed modulator and high-resolution speed controller (Devices for Science). The infusion rate was identical to that for the maintenance dose of aminophylline (fig. 1). After this 30-minute saline infusion, the loading dose of aminophylline (2.8, 5.6, 8.4 or 11.2 mg/kg total body weight) was begun from the second syringe by adjustment of a stopcock. At the completion of the loading dose (30 minutes), the infusion rate was reduced to the maintenance level (0.45, 0.9, 1.35 or 1.8 mg/kg estimated ideal body weight per hour). This was continued for 90 minutes; saline was then reinstituted for the remaining 60 minutes of the study (fig. 1). The loading doses were calculated on the basis of total body weight10 and the maintenance doses on the basis of estimated ideal body weight using a standard dosage schedule.4 After the fourth study, each subject was questioned regarding the protocol. All had assumed that aminophylline was begun when the pump was initially activated (saline infusion). The aminophylline (Aminophyllin Injection,* 25 mg/ml, lot 778-843, 85.3% anhydrous theophylline by weight) was diluted with 0.9% saline to achieve concentrations of 2, 4, 6 or 8 mg/ml. Thus, with successively higher doses, infusion rates and the delivered fluid volumes remained the same for an individual subject except for minor adjustments. Additional saline required to maintain i.v. needle patency averaged 345 ± 85 ml (SD) during the 4-hour study.

*Kindly provided by Elliott N. Schubert, Ph.D., Searle Laboratories, Chicago, Illinois.
| Desired plateau plasma theophylline (µg/ml) | 5.0  
| Actual plateau plasma theophylline (µg/ml)* | 4.5 ± 0.2 | 10.0  
| B† | I‡ | B | I | 10.0 ± 0.5 |
| Heart rate (beats/min) | 53 ± 3 | 53 ± 3 | 56 ± 4 | 56 ± 3 |
| Systolic blood pressure (mm Hg) | 120 ± 4 | 121 ± 4 | 117 ± 3 | 120 ± 3 |
| Diastolic blood pressure (mm Hg) | 77 ± 5 | 80 ± 4 | 77 ± 2 | 79 ± 2 |
| Mean arterial pressure (mm Hg) | 92 ± 4 | 93 ± 4 | 91 ± 2 | 92 ± 2 |
| QS2 index (msec) | 569 ± 10 | 552 ± 11| 560 ± 9 | 544 ± 7|
| Left ventricular ejection time index (msec) | 434 ± 4 | 420 ± 6| 434 ± 8 | 418 ± 5|
| PEP/LVET | 135 ± 7 | 131 ± 6 | 128 ± 4 | 126 ± 4 |
| Ejection fraction | 0.76 ± 0.02 | 0.72 ± 0.02 | 0.75 ± 0.03 | 0.77 ± 0.03 |
| Glucose (mg/dl) | 83 ± 3 | 78 ± 3 | 81 ± 2 | 83 ± 2 |
| Free fatty acids (µEq/l) | 506 ± 36 | 604 ± 65 | 496 ± 67 | 788 ± 90* |
| Insulin (µU/ml) | 14 ± 3 | 11 ± 2 | 9 ± 1 | 11 ± 2 |
| Epinephrine (pg/ml) | 26 ± 5 | 40 ± 10 | 30 ± 3 | 57 ± 11 |
| Norepinephrine (pg/ml) | 160 ± 19 | 197 ± 19 | 178 ± 4 | 244 ± 21 |

Values are mean ± SEM.

**Actual plateau concentration of theophylline was determined from the average of plasma concentrations at 60, 90, and 120 minutes.

‡Basal values before theophylline infusion were obtained from the average of values at -15 and 0 minutes for each individual except for systolic time intervals and ejection fraction, which represent the average of values at -30 and 0 minutes.

§Infusion values are the average of data for each individual obtained during the final 30 minutes of the theophylline infusions (90-120 minutes). Comparisons of infusion vs basal values by paired t test:

††p < 0.05.

§§p < 0.025.

+++p < 0.01.

*†p < 0.005.

can Society of Echocardiography. Ejection fraction (EF) was estimated according to the equation:

\[
\frac{LVd^3 - LVd^3}{LVd^3}
\]

Aliquots of blood samples for theophylline, glucose, insulin and FFA were added to chilled, heparinized tubes on ice (EDTA rather than heparin was used for FFA), and centrifuged at 4°C for 15 minutes; the plasma was separated and frozen at -20°C until assayed. Aliquots of whole blood for epinephrine and norepinephrine were added to chilled tubes on ice containing EGTA and reduced glutathione, centrifuged at 4°C; the plasma was separated, recentrifuged and likewise stored at -20°C until assayed.

Theophylline assay was performed by high-pressure liquid chromatography using a Micromeritics model 750 solvent delivery system equipped with a model 730 Universal Injector and model 786 variable wavelength detector (Micromeritics Instrument Corporation). Theophylline was extracted from 1-ml aliquots of plasma to which beta-hydroxyethyl-theophylline (10 µg) had been added as an internal standard using 10 ml of ether:dichloromethane:isopropanol (6:4:1 by volume with 1.5% isoamyl alcohol), evaporated to dryness under nitrogen, and redissolved in mobile phase for injection onto a µBondapak C18 reversed phase column (Waters Associates) with an acetonitrile:water (7:93 by volume) mobile phase and a flow rate of 2 ml/min. Absorbance was recorded at 280 nm. Concentrations of unknown samples were determined by peak height ratios of theophylline to internal standard from a standard curve subjected to linear regression analysis. Water was deionized (Milli-Q-Water System, Millipore) and reagents were analytical grade (Burdick and Jackson). The latter were filtered through a 0.45-µ millipore filter before use. The intraassay coefficient of variation for the assay was 10.4, 4.6 and 4.2% at 0.1, 1 and 20 µg/ml, respectively. The interassay coefficient of variation is approximately 6% using a quality-control sample of 3.5 µg/ml.

Plasma protein binding of theophylline was determined in triplicate by equilibrium dialysis (37°C) of 1.2 ml blank plasma against an equal volume of 0.15 M phosphate buffer (pH 7.4) containing 25 µg/ml theophylline using rigid, clear acrylic cells (Fisher Scientific Co.) separated into two 1.5-ml compartments by a cellophane membrane (Union Carbide Corp.). A single concentration was used, based on preliminary experiments showing no effect of concentration on binding. At equilibrium (12-16 hours as determined by preliminary studies), 1-ml aliquots of buffer and plasma were frozen for theophylline assay.

Plasma glucose was measured by the glucose oxidase method, plasma insulin by radioimmunoassay using a modification of the double antibody technique of Morgan and Lazarow, and FFA by a modification of the method of Dole. Plasma norepinephrine and epinephrine were measured with the specific and sensitive single-isotope enzymatic assay described by...
Peuler and Johnson. In our laboratory, this method has an intraassay coefficient of variation of 6% for norepinephrine (175 pg/ml range) and 5% for epinephrine (75 pg/ml range). The interassay coefficient of variation is 13% and 10%, respectively, for norepinephrine and epinephrine.

Statistical analysis of the data in table 1 was conducted using the paired t test (two-tailed probabilities). The data, as shown in figures 1 and 4, were subjected to a three-factor factorial design analysis of variance (ANOVA) using the SPSS computer program. This design for ANOVA tests the significance of each factor while simultaneously controlling for the variation in the remaining two factors. For example, the test of significance of response over time controls for variation in subject response and dose response. The data in figures 2, 3, and 5 were analyzed by a single-factor ANOVA with repeated measures. In this analysis, Dunnett’s test was used to compare results at the 10-, 15-, and 20-μg/ml dose levels with those at 5 μg/ml.

Values are mean ± SEM. An α value of 0.05 was considered maximum for statistical significance and significance levels of ANOVA were based on the computed F values with appropriate degrees of freedom.

Results

Concentration of Theophylline in Plasma

Concentrations of theophylline in plasma increased steadily during the loading infusion of aminophylline and achieved peak levels at 30 minutes of 6.8 ± 0.4, 11.5 ± 0.8, 15.7 ± 1.3, and 24.9 ± 3.7 μg/ml for the low-dose, the two intermediate-dose, and the high-dose protocols, respectively (fig. 1). There was considerable variation between subjects, which was most marked in the final study. Concentrations at 30 minutes in that study ranged from 13.6 to 35.7 μg/ml, but in five of the six subjects the 30-minute concentration exceeded 20 μg/ml. The one subject with low initial levels achieved an average plasma concentration of 18.0 μg/ml during the maintenance infusion. One subject developed nausea without emesis 5 minutes after the end of the loading infusion and had a peak theophylline concentration of 34 μg/ml. The maintenance infusion of aminophylline was stopped and his data were excluded from the analysis of results for the 20 μg/ml goal plasma level. The other subject with a high peak concentration (36 μg/ml) suffered no untoward effects.

During the maintenance infusion of aminophylline, plateau concentration theophylline averaged 4.5 ± 0.2, 10.0 ± 0.5, 14.0 ± 0.5 and 20.0 ± 1.2 μg/ml between 60 and 120 minutes (fig. 1, table 1) and very closely approximated the desired plasma levels of 5, 10, 15, and 20 μg/ml. Plasma levels were reasonably stable during this period and adequately simulated steady-state conditions. At the termination of the 60-minute saline placebo infusion, theophylline concentrations fell only slightly, to 4.0 ± 0.2, 8.9 ± 0.4, 12.5 ± 0.5 and 18.9 ± 0.6 μg/ml. The plasma concentration-time curves for each dose level were significantly different with respect to both dose and time (p < 0.001), but not with respect to subject. Plasma protein binding differed very little between the studies and averaged 45.1 ± 0.6%, 46.3 ± 1.9%, 48.0 ± 0.8% and 48.5 ± 1.5% for the four dose levels. These values are somewhat lower than those previously reported except for those observed by Fleetham et al. The discrepancy may reflect differences in methods, particularly a longer incubation period in our study.

Heart Rate and Blood Pressure

Heart rate was unchanged at desired plasma theophylline levels of 5 and 10 μg/ml, but increased significantly with time at levels of 15 and 20 μg/ml in a dose-dependent fashion (F3,34 = 42.018, p < 0.001). The increase in mean heart rate was apparent by the end of the loading infusion and persisted for at least 60 minutes after the maintenance infusion was discontinued. During the final 30 minutes of the aminophylline maintenance infusion at these two higher dose levels, heart rate increased an average of 8.2 ± 1.4 and 26.9 ± 11.6 beats/min above baseline values (fig. 2).

Systolic blood pressure showed changes similar to those observed for heart rate and increased significantly during the 15- and 20-μg/ml plasma level infusions (F3,34 = 18.034, p < 0.001). During the final 30 minutes of the infusion at these two goal plasma levels, systolic blood pressure increased an average of 6.8 ± 2.2 and 15.0 ± 4.7 mm Hg (fig. 2). Diastolic blood pressure did not change significantly after the aminophylline infusions. However, small increases were noted, and at the 20-μg/ml plasma level, diastolic blood pressure increased an average of 6.3 ± 2.8 mm Hg during the 90-120-minute period. Mean arterial pressure tended to increase with time, mainly during the high-dose aminophylline infusion.

Systolic Time Intervals

Both the QS2 index and the LVET index (table 1)
decreased significantly with time during the aminophylline infusions ($F_{3,167} = 4.524$ and $F_{3,167} = 8.613$, both $p < 0.001$), but the effect did not appear to be related to theophylline concentration. Thus, the average change from baseline in $QS_{2}$ index measured at the 90- and 120-minute points was $-17.6 \pm 5.1$, $-16.2 \pm 3.0$, and $-17.9 \pm 3.8$ msec for the 5-, 10- and 15-$\mu g/ml$ plasma levels, respectively (all $p < 0.025$). The average value at the 20-$\mu g/ml$ level was $1.9 \pm 18.5$ msec. This mean value includes data for one subject whose heart rate was 125 beats/min at the 120-minute time point (well outside the range used to determine the heart rate correction factor). If his result is excluded, the mean value for the remaining four subjects is $-15.9 \pm 6.2$ msec ($p < 0.1$). The corresponding values for LVET index were $-13.7 \pm 4.8$, $-23.3 \pm 3.6$, and $-18.3 \pm 4.3$ msec for the 5-, 10- and 15-$\mu g/ml$ plasma levels, respectively (all $p < 0.05$), with an average value of $-2.0 \pm 13.6$ msec at the highest plasma level. If the same subject is excluded, the average for the remaining four subjects is $-15.5 \pm 1.9$ msec ($p < 0.005$). The PEP index and the ratio of PEP to LVET (except at the 15 $\mu g/ml$ plasma level) were not influenced by the aminophylline infusions.

**Left Ventricular Ejection Fraction**

Consistent with a previous study, estimates of left ventricular EF obtained from echocardiographic sys-
tolic and diastolic internal cavity dimensions were unchanged during the aminophylline infusions (table 1).

**Plasma Glucose and Free Fatty Acids**

During the low-dose aminophylline infusion (5 $\mu g/ml$ goal plasma level), plasma glucose concentrations tended to fall slightly with time. In contrast, during the intermediate- and high-dose infusions (15- and 20-$\mu g/ml$ goal plasma levels), glucose concentrations tended to increase slightly. The analysis of variance for a dose effect was significant ($F_{3,212} = 16.605$, $p < 0.001$). However, comparisons of glucose values during the final 30 minutes of the infusions did not reveal significant differences from baseline values (table 1) or from the values obtained with the low-dose infusions (fig. 3).

The levels of FFAs in plasma increased significantly in a dose-dependent ($F_{3,212} = 7.918$, $p < 0.001$) and time-dependent ($F_{3,212} = 13.025$, $p < 0.001$) manner during the aminophylline infusions. At the highest dose, FFAs increased twofold, from $494 \pm 59 \mu Eq/l$ at baseline to an average of $993 \pm 91 \mu Eq/l$ during the final 30 minutes of the aminophylline infusion ($p < 0.005$); however, increases were already nearly at maximal levels by the end of the loading infusion. The response to aminophylline can also be appreciated if the average change from baseline at 90 and 120 minutes for each dose level is compared (fig. 3). Except for two subjects at the 5-$\mu g/ml$ plasma level of theophylline, all subjects had net increases in plasma FFAs above baseline during the four aminophylline infusions.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Changes in heart rate and blood pressure. Mean differences between the average of values during the final 30 minutes of the aminophylline infusion (90-, 105- and 120-minute values) and the baseline values (average of -15- and 0-minute values) are shown for each dose. The change in heart rate and systolic blood pressure from baseline was significantly related to aminophylline dose ($F_{3,15} = 6.584$ and $F_{3,15} = 7.685$, both $p < 0.005$). At the high-dose level (20-$\mu g/ml$ goal plasma theophylline level) the net increase in heart rate and in systolic blood pressure was greater than the increase observed at the low dose level (5-$\mu g/ml$ goal). The changes in diastolic blood pressure were not significant.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Changes in plasma glucose, free fatty acids and insulin. Mean differences between the average of values during the final 30 minutes of the aminophylline infusion (90- and 120-minute values) and the baseline values (average of -15- and 0-minute values) are shown for each dose of aminophylline. The change in free fatty acids and insulin levels from baseline was significantly related to dose ($F_{3,15} = 10.953$, $p < 0.001$, and $F_{3,15} = 3.415$, $p < 0.05$). The net increase in free fatty acids was significantly greater at the two intermediate (10- and 15-$\mu g/ml$ goal plasma theophylline levels) and at the high-dose (20-$\mu g/ml$ goal) than at the low-dose level (5-$\mu g/ml$ goal). A similar comparison for insulin was significant only at the highest dose level.
Plasma Insulin

Plasma insulin levels tended to fall during the low-dose aminophylline infusion, but increased slightly with the higher doses in a dose-dependent manner ($F_{3,212} = 8.112$, $p < 0.001$). Insulin levels changed significantly with time ($F_{9,212} = 2.160$, $p < 0.02$). However, the average change from baseline during the final 30 minutes of the infusion (table 1) was small ($-3 \pm 22, 2 \pm 1, 2 \pm 2$, and $6 \pm 3 \mu U/ml$) and was only significant for the 10-μg/ml dose level ($p < 0.05$). These differences were consistent with a small effect of theophylline concentration on plasma insulin (fig. 3).

Plasma Epinephrine and Norepinephrine

 Plasma concentrations of epinephrine and norepinephrine increased in a dose-dependent manner (table 1, fig. 4). At the highest dose, plasma epinephrine

![Graph](https://example.com/graph.png)

**Figure 4.** Changes in plasma epinephrine and norepinephrine. Plasma epinephrine (left) and norepinephrine (right) increased with aminophylline dose ($F_{3,212} = 23.970$ and $F_{3,212} = 14.142$, both $p < 0.001$) and with time ($F_{3,212} = 21.546$ and $F_{3,212} = 8.516$, both $p < 0.001$). Dotted vertical lines indicate period of aminophylline infusion.
increased fourfold, from 29 ± 4 pg/ml at baseline to 105 ± 14 pg/ml during the final 30 minutes of the aminophylline infusion (p < 0.01). During the same period, norepinephrine increased from 190 ± 18 to 312 ± 51 pg/ml (p < 0.05). Comparison of the average change from baseline for plasma epinephrine and norepinephrine during the final 30-minute period of the aminophylline infusion suggests that the relationship to aminophylline dose is more consistent for epinephrine than for norepinephrine (fig. 5).

Discussion

Methylxanthines, such as caffeine and theophylline, are potent stimulants of the central nervous system and have also been reported to stimulate the release of epinephrine and norepinephrine from the sympathoadrenal system. They exert both positive inotropic and chronotropic effects on the heart, while peripheral vascular resistance is reduced, regardless of any change in arterial blood pressure. The metabolic effects include stimulation of gastric acid secretion and an increase in plasma FFAs. The major therapeutic application of theophylline is to promote bronchial smooth muscle relaxation in the clinical setting of acute or chronic bronchospasm, but it also has a diuretic effect that can be useful in the treatment of heart failure, particularly acute pulmonary edema. This effect on kidney function is thought to be secondary to an increase in renal blood flow. These cardiovascular, metabolic and pulmonary responses have generally been attributed to inhibition of the degradation of cyclic AMP. However, other mechanisms have been suggested.1

At the highest of four doses, the i.v. administration of aminophylline to six healthy male subjects resulted in a mean plateau plasma theophylline level of 20 μg/ml, a 48% increase in resting heart rate, and an 11% increase in systolic blood pressure, without a significant change in diastolic blood pressure. A positive inotropic effect was also observed, but was statistically significant only at submaximal aminophylline dose levels. Plasma FFA levels increased twofold, with smaller increases in plasma glucose and insulin. These dose-dependent cardiovascular and metabolic effects occurred in association with a 262% increase in plasma epinephrine and a 64% increase in plasma norepinephrine during the high-dose infusion, suggesting that the cardiovascular and metabolic effects of theophylline may be mediated in part by stimulation of the sympathetic nervous system.

The chronotropic and pressor response to acute aminophylline administration was dose-dependent. At low plasma concentrations of theophylline (5 and 10 μg/ml), heart rate and blood pressure did not change significantly; however, at intermediate and high concentrations (15 and 20 μg/ml), mean heart rate and systolic blood pressure increased. This suggests a threshold between 10 and 15 μg/ml for the heart rate and blood pressure effects of theophylline. Diastolic blood pressure was not markedly altered. Using loading and maintenance doses of aminophylline to produce three consecutive plasma theophylline concentration plateaus of 5, 10 and 20 μg/ml during a 180-minute study period, Ogilvie et al.4 could not demonstrate a dose-response relationship for heart rate and blood pressure in five subjects.4 However, the duration of each plateau was only 30 minutes.

In contrast to the chronotropic and pressor responses, the positive inotropic response to aminophylline demonstrated in this study did not seem to be dose-dependent. Since the decreases in QS2 index and LVET index were similar at 5-, 10- and 15-μg/ml plasma theophylline concentrations, very low concentrations of this methylxanthine can apparently enhance cardiac contractility, and the maximum response is easily achieved at concentrations within the usual therapeutic range (10–20 μg/ml). Systolic time intervals provide only an indirect assessment of cardiac performance. However, the QS2 index is thought to be a sensitive and specific measure of increased cardiac inotropy due to pharmacologic agents.25–7 Since the LVET index may be decreased by a decrease in preload, by left ventricular muscle failure, and by both positive and negative inotropic agents, the LVET index also reflects changes in inotropic state, but is less specific for drug effects than the QS2 index.

The apparent difference in cardiac sensitivity to the chronotropic and the inotropic effects of theophylline

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Change in plasma epinephrine and norepinephrine. Changes from baseline were determined as in figure 3 and were significantly related to aminophylline dose for both plasma epinephrine (F1,15 = 8.617, p < 0.005) and norepinephrine (F1,15 = 3.801, p < 0.05). The net increases in epinephrine at the intermediate- (15-μg/ml goal) and high- (20-μg/ml goal) dose levels were greater than those observed at the lowest dose. For norepinephrine, this comparison was significant only at the high-dose level.
suggests that the mechanism of action may differ in pacemaker tissue compared with myocardial tissue. Alternatively, the parasympathetic nervous system, which exerts dominant control over heart rate through vagal innervation, may inhibit the chronotropic action of theophylline. Thus, slowing of the heart by the parasympathetic nervous system could attenuate an increase in heart rate caused by a direct effect of theophylline on the sinus node or by a theophylline-induced increase in sympathetic nervous system activity. Although basal parasympathetic tone may be sufficient to exert this homeostatic response, theophylline could stimulate parasympathetic as well as sympathetic activity.¹

Elevations in plasma FFAs, which suggest increased lipolytic activity, occurred promptly after initiation of the aminophylline infusions. The elevations were dose-related and were the same magnitude observed in studies using loading doses of 6–9 mg/kg.⁵,⁷,¹⁴,¹⁵ Aminophylline-induced elevations in FFAs are markedly attenuated by concomitant administration of propranolol,¹⁴ providing further evidence that the effect of aminophylline is at least partially mediated by catecholamines. The effect of theophylline on plasma glucose levels were small.⁵,¹⁴,¹⁵ The insulin response observed in this study was also small and seemed to parallel the change in plasma glucose. Ensinck et al.¹⁴ observed maximal increases of about 250% in plasma insulin after a 500-mg infusion of aminophylline. Others have found either no significant change or a comparable increase, allowing for the difference in dose.¹⁵

The average maximal increase of plasma epinephrine and norepinephrine was approximately 100 pg/ml, the same for each catechol. Since secretion of epinephrine from the adrenal gland normally exceeds that of norepinephrine by two- to fourfold,²⁸,²⁹ the source of the increase in plasma norepinephrine is probably from postganglionic sympathetic neurons rather than the adrenal medulla. The findings in this study of increased circulating plasma epinephrine and norepinephrine after aminophylline are not unexpected. Intravenous aminophylline (500 mg) is associated with increases in the urinary excretion of epinephrine and norepinephrine.⁷ Oral caffeine (250 mg) also increases circulating plasma epinephrine and norepinephrine, as well as urinary metanephrine and normetanephrine.⁸ However, the apparent stimulation of the sympathoadrenal system is clearly a dose-related phenomenon. This has not been previously reported in human studies.

There are several mechanisms by which theophylline could have caused an increase in plasma catecholamines. The diuretic effect of aminophylline may have caused volume contraction and reflex stimulation of the carotid baroreceptor mechanism. However, since systolic blood pressure actually increased during the infusions of aminophylline with little change in diastolic pressure, it appears unlikely that the increase in catecholamines could be explained by carotid baroreceptor activation with a resultant increase in sympathetic tone. The possibility that uptake or metabolism of catecholamines is inhibited by theophylline is also not excluded by this study; however, the results from previous studies of caffeine⁶ and aminophylline,⁷ which reported increased urinary catecholamine metabolite excretion, make this explanation less likely. Although plasma norepinephrine and FFAs reached an apparent plateau by the end of the loading dose of aminophylline, epinephrine continued to increase progressively during the maintenance infusion. Whatever the mechanism of epinephrine release under the conditions of this study, it is apparently influenced by time as well as the actual plasma level of theophylline.

Direct stimulation of sympathetic nervous system activity is also possible. Although the concentrations used were very high (0.5–5.5 mM), theophylline and aminophylline have been shown to stimulate the release of catecholamines from isolated perfused bovine¹⁰ and feline adrenal glands.⁹ Some other experiments have suggested a central nervous system mechanism for methylxanthine-stimulated adrenal catecholamine release. Adrenal dopamine content, which increases with catecholamine secretion, increased 50% after theophylline administration to rats, but this effect was not observed in animals with prior C-7 spinal cord transection.³⁰ Other studies in the rat and guinea pig indicate that administration of theophylline results in both central (brain and brainstem) and peripheral (heart) neuronal release of norepinephrine.¹¹,¹²

Several molecular mechanisms for the cardiac, hemodynamic and metabolic effects of methylxanthines have been proposed,¹ including antagonism of a cell surface receptor for adenosine.³³⁻³⁵ Theophylline and related compounds such as caffeine can inhibit phosphodiesterase and thereby increase the intracellular levels of cyclic AMP. Although phosphodiesterase inhibition is widely invoked as the mechanism of action of theophylline, the extent to which this occurs and its importance at therapeutic plasma concentrations is open to question. Since plasma protein binding of theophylline averages about 50% (47% in this study), the free concentration of theophylline at therapeutic levels is 4–10 µg/ml (22–55 µM). At these concentrations in vitro, theophylline is a very weak inhibitor of phosphodiesterase activity and is not associated with an elevation in tissue levels of cyclic AMP.³⁹,⁴⁰ Although 10 µM theophylline relaxed carbachol contracted bovine tracheal muscles by 85–95%,³⁹ similar concentrations had little or no effect in isolated heart muscle preparations.⁴⁰,⁴¹ Perfusion of the canine sinus node artery with low concentrations of aminophylline (1–10 µg/ml) did not cause a change in heart rate, but a much higher concentration (100 µg/ml) resulted in an immediate tachycardia.¹¹

Rutherford et al.¹³ observed positive inotropic, chronotropic and pressor responses after i.v. aminophylline, 10 mg/kg, in the conscious dog. The positive chronotropic response was abolished and the inotropic response was markedly attenuated by β-adrenergic blockade with propranolol or chronic treatment with reserpine; the increase in systemic vascular resistance and mean arterial pressure was abolished by α-adren-
ergic blockade with phenolamine. In contrast to systemic administration, direct infusion of aminophylline into the iliac artery resulted in vasodilatation. These investigators and others have proposed that the cardiac and hemodynamic effects of theophylline may be explained, at least in part, by the release of endogenous catecholamines. Our findings that plasma catecholamines increase during theophylline administration in man also supports this hypothesis.

The physiologic threshold for an increase in heart rate due to circulating plasma epinephrine is 50–100 pg/ml, while that for an increase in systolic blood pressure and blood glycerol is 75–125 pg/ml. Levels within this range were achieved in this study. The thresholds for alterations in plasma glucose, insulin and diastolic blood pressure are considerably above these levels. Plasma norepinephrine levels in excess of 1800 pg/ml are required to produce hemodynamic and metabolic effects during i.v. infusion of norepinephrine. However, since only a small proportion of neuronally released norepinephrine spills into plasma, even a small increment in plasma norepinephrine may reflect significant sympathetic nervous system activation with sufficient stimulation of postsynaptic receptors at the site of release to have measurable hemodynamic and metabolic effects. Thus, our present results are consistent with stimulation by theophylline of neuronal and adrenomedullary catecholamine release, which may have contributed to the observed chronotropic, inotropic, pressor and lipolytic effects.

The precise clinical significance of the sympathoadrenal stimulation by theophylline in this study is unknown. As with caffeine, chronic administration of theophylline preparations may result in an adaptation to these effects. Nevertheless, in the setting of acute administration to critically ill patients, stimulation of central and peripheral catecholamine release by theophylline may account for its troublesome cardiac toxicity. This aspect of its pharmacology merits further investigation.

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Nucleotide Degradation and Functional Impairment During Cardioplegia: Amelioration by Inosine

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SUMMARY The degradation of adenine nucleotide levels and impairment of functional recovery associated with exposure to hypothermic (20°C) cardioplegia was studied in 84 isolated working rat hearts. After a 1-hour control period, hearts were exposed to 1 hour of cardioplegia that consisted of increasingly longer periods of cardioplegic solution (CPS) infusion (30 seconds and 10, 30, and 60 minutes), followed by increasingly shorter periods of global ischemia (59½ minutes and 50, 30 and 0 minutes). Hearts were then reperfused for 1 hour with control perfusate, during which recovery of cardiac output was monitored. Additional hearts were freeze-clamped at various points in the protocols to determine adenine nucleotide levels (ATP, ADP, AMP and their sum TAN). Exposure to increasingly longer periods of CPS perfusion resulted in proportionally greater degradation of nucleotides and poorer recovery of cardiac output. Inclusion of inosine in the CPS reduced the degradation of ATP and TAN and improved functional recovery. Addition of inosine to the recovery perfusate as well as the CPS further improved nucleotide levels and recovery of cardiac output. These results suggest that washout of nucleotide degradation products in the CPS or reperfusion prevents their salvage for nucleotide resynthesis and impairs functional recovery from cardioplegia.

PROTECTION of the myocardium during cardiopulmonary bypass and ischemic arrest is a major problem of cardiac surgery, and no ideal method has been found. Cold cardioplegic solutions (CPS) are widely used for this purpose, but the characterization of the ideal composition and method of administration is incomplete.

A major objective of myocardial protection is conservation of the adenosine nucleotide pool during the ischemic period. At any moment, myocardial ATP content is a function of its rates of synthesis and degradation. Although hypothermic cardioplegia greatly reduces energy use during ischemia,1-4 ATP degradation still outstrips production and nucleotide degradation products such as adenosine, inosine and hypoxanthine accumulate in the myocardium.5,6 These constitute a purine base pool available for resynthesis of ATP during the postischemic recovery period. Failure to recover normal cardiac function after cardiopulmonary bypass correlates well with the inability to resynthesize normal levels of ATP.1-4,7,8 However, this may be a consequence of the loss of ATP precursors rather than...
Effect of intravenous aminophylline on plasma levels of catecholamines and related cardiovascular and metabolic responses in man.
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