Stimulation of endogenous catecholamine release by theophylline: a proposed additional mechanism of action for theophylline effects

Martin D. Higbee, Pharm.D., Manju Kumar, Pharm.D., and Stanley P. Galant, M.D., Salt Lake City, Utah, Ann Arbor, Mich., and Long Beach, Calif.

Therapeutic response to theophylline in asthma is generally attributed to its effect in increasing intracellular 3',5' cyclic adenosine monophosphate (cAMP) by competitive inhibition of cAMP phosphodiesterase. However, because of discrepancies between therapeutic serum theophylline concentration achieved clinically and those required for in vitro phosphodiesterase inhibition, we explored the possibility that theophylline may act through adrenomedullary secretion of catecholamines. Five healthy, nonasthmatic male and female adults were studied with a double-blind, randomized, crossover protocol. Theophylline (5 mg/kg) and placebo were administered in a capsule dosage form. Plasma catecholamines epinephrine (E), norepinephrine (NE), and dopamine (DA) were measured by a radioenzymatic assay at baseline and after administration of theophylline at 1, 2, and 3 hr. Significant differences between theophylline- and placebo-treated groups (p < 0.05) were seen at 3 hr for mean percentage increase over baseline with E (120.7 ± 25.3%) and NE (48.0 ± 17.9%) after theophylline therapy (mean peak level 7.2 ± 0.48 pg/ml). Epinephrine plasma concentration was significantly greater (p < 0.001) at 3 hr compared with baseline (105 ± 16 vs 56 ± 18 pg/ml), while NE (448 ± 57 vs 320 ± 36 pg/ml) did not attain significance (p = 0.136). A significant correlation (r = 0.66) was found between the percentage increase over baseline for E (r = 0.58) and NE (r = 0.66) and serum theophylline levels. DA was not significantly increased at any time period. Thus theophylline in clinically relevant concentration appears to stimulate adrenomedullary secretion of catecholamine. Whether this is an important mechanism of action in asthma or explains some side effects of theophylline remains to be determined. (J ALLERGY CLIN IMMUNOL 70:377, 1982.)

The methylxanthines, caffeine, theophylline, and theobromine, have several pharmacologic actions, of which their ability to relax bronchial smooth muscle has great value in the treatment of chronic obstructive pulmonary disease. Theophylline is the most effective of the methylxanthines in this regard. Clinically, it is used either alone or in combination with beta-adrenergic agonists. The pharmacologic basis of this combination is thought to be enhancement of the intracellular concentration of cAMP. The methylxanthines, particularly theophylline, are competitive inhibitors of the enzyme phosphodiesterase, which catalyzes the hydrolysis of cAMP to inactive 5' AMP, whereas the catecholamines increase the concentration of cAMP through stimulation of adenylyl cyclase. The enzyme responsible for cAMP synthesis from adenosine triphosphate. However, the therapeutic concentration of theophylline achieved in vivo (50 μM or 9 to 18 μg/ml) inhibits phosphodiesterase activity in many tissues by only 5% to 10% and doubt as to this inhibition as the only mechanism of action has been suggested. Consequently, to explain this disparity between in vivo effects of similar concentrations, attention has been focused in recent years on other potential mechanisms of action of methylxanthines. Horrobin et al. demonstrated that methylxanthines exhibited prostaglandin antagonism, with no effect on synthesis, and proposed the clinical effects
Abbreviations used

- CAMP: 3',5' Cyclic adenosine monophosphate
- DA: Dopamine
- E: Epinephrine
- NE: Norepinephrine

of theophylline are due to this antagonism. Fredholm, on the other hand, suggested that theophylline, in clinically achievable concentrations, antagonizes the effects of adenosine. He therefore proposed that this antagonism is responsible for the observed pharmacologic actions of theophylline more so than inhibition of cAMP hydrolysis. Kolbeck et al. attributed theophylline-induced smooth-muscle relaxation to increase of calcium uptake into smooth-muscle cells. However, other studies suggest a role for theophylline-induced catecholamine release as an additional factor in the mediation of the pharmacologic actions of theophylline.

Animal studies with theophylline and caffeine have demonstrated a stimulatory effect of the xanthines on the adrenal medulla to cause a release of catecholamines, specifically E. This adrenomedullary release of catecholamines was noted to be independent of extracellular calcium concentrations. However, the theophylline concentration used in these models was 10 to 20 times greater than that usually employed clinically.

To explore the stimulating effect of the methylxanthines on the adrenal medulla, earlier studies in human subjects were performed by quantitative determinations of NE and E in the urine after a caffeine or theophylline challenge. These studies demonstrate a statistically significant increase in urinary concentration of E and NE after the ingestion of methylxanthine. Atuk et al. demonstrated an increased excretion of urinary E (p < 0.01) and NE (p < 0.05) after a 500 mg infusion of aminophylline, which according to the author reflected an augmented concentration of the amines in the plasma. Robertson et al. reported that caffeine ingestion is one of the most potent stimuli for adrenomedullary catecholamine release. Ingestion of 250 mg of caffeine by overnight-fasted supine subjects resulted in caffeine plasma concentrations averaging 10 μg/ml and increased E and NE concentration by 75%, respectively. The effect of theophylline on altering plasma catecholamines has only recently been addressed in man. The availability of a sensitive single-isotope radioenzymatic assay for plasma catecholamines has facilitated quantitative analysis of E and NE. This makes such a study a logical next step in the evaluation of alternative mechanisms of action for theophylline.

The objective of this pilot study was to determine whether theophylline in clinically recommended doses causes an increase of plasma catecholamines, which might provide an additional mechanism of action for theophylline in asthma and explain some of the pharmacologic adverse effects encountered with theophylline use.

MATERIALS AND METHODS

Subject selection

Five healthy, nonasthmatic subjects were selected for the study. There were two men and three women with an average age of 27 yr (range 24 to 32 yr). Health status and suitability to participate in the study were determined by medical history and a physical examination conducted by the physician investigator. Objective evaluation was completed by the following tests: serum electrolytes, blood urea nitrogen, serum glucose, complete blood count with differential, T₄ and T₂ and free thyroxine index, and urinalysis.

Exclusion criteria

The following exclusion criteria were used: (1) ingestion of coffee, tea, cola drinks, or any chocolate-containing food 3 days prior to the study, (2) history of cigarette smoking, (3) ingestion of alcoholic beverages during the 3 days prior to the study, (4) ingestion or inhalation of drugs or drug combination products containing α-methyldopa or isoproterenol, since these agents may interfere with assay, (5) history of ingesting any sympathomimetics or bronchodilators, (6) history of ingesting any antihypertensive, antidepressant, antianxiety, diuretic, sedative-hypnotic, psychoactive medications, or exposure to any contrast-media tests utilizing radioactive iodine, and (7) history of hypertension, cardiovascular disease, asthma or lung disease, diabetes mellitus, hepatitis or unexplained jaundice, hypothyroidism and hyperthyroidism, or seizure disorders.

Study design

A double-blind, randomized, crossover trial was conducted on two separate days at the University Hospital Clinical Research Center in Salt Lake City. There was a washout interval of 3 days between the 2 study days. On the first day, the subjects were randomly assigned to placebo or theophylline by means of a random number table.

On each of the days of study, each individual arrived 1 hr before blood sampling for the placement of a catheter (No. 20 Angio Cath; Deseret Pharmaceuticals). The subjects provided a urine sample for qualitative analysis of methylxanthines to rule out any evidence of their ingestion. After voiding, the subjects remained supine in nonstimulating surroundings for 1 hr to recover from any minor stress associated with the cannulating procedure. Thereafter, 5 ml of blood were drawn directly from the infusion set into an evacuated tube for determination of baseline catechol-
amine. Subsequently, a single dose of 5 mg/kg theophylline (Aminophyllin; Searle Laboratories) prepared in a gelatin capsule was administered to the subjects who were to receive the drug, and those receiving the placebo swallowed a lactose-containing capsule that was indistinguishable from the theophylline capsule. Blood samples for theophylline and catecholamine determinations were obtained 1, 2, and 3 hr after ingestion of the theophylline or placebo capsule. Subjects receiving drug or placebo on the first day received the reciprocal preparation on the second study day. The time of the blood sample collection each study day was consistent. The subjects assumed a recumbent position during the entire study. Vital signs (blood pressure, temperature, pulse, and respiration) were monitored hourly by the nursing staff. In addition, subjects were monitored for side-effects of theophylline such as nausea, vomiting, restlessness, irritability, tachycardia, and feelings of anxiety. The exact time of blood sampling was recorded by the nursing staff. The blood samples were immediately placed in an ice bath and centrifuged within 15 to 20 min to separate the plasma from the cells. Plasma was decanted into screw-capped glass tubes (Pyrex No. 9826) and promptly frozen at -70°C to avoid the loss of catecholamines by oxidation. Samples were analyzed for catecholamine content by the radioenzymatic procedure described by Peuler and Johnson19 (Cat-A-Kit; Upjohn Diagnostics). The sensitivity of the assay is in the range of 2 to 5 pg/50 μl (data not shown). Significance between theophylline and placebo treatments were not noted at any time after placebo administration. However, a significant correlation (p < 0.05) was found between the percentage increase above baseline for E (r = 0.58) and NE (r = 0.66) and the serum theophylline level (Fig. 4).

RESULTS

The use of the commercially available Cat-A-Kit in our laboratory resulted in a 5% variability between duplicate samples and controls provided in the kit. The mean (± SEM) plasma E, NE, and DA levels (pg/ml) after theophylline and placebo administration are depicted in Table I. The mean (± SEM) theophylline level (μg/ml) is also shown at 1, 2, and 3 hr after drug administration. E serum concentration was significantly elevated (p < 0.001) at 3 hr as compared with baseline (105 ± 16 vs 56 ± 18 pg/ml), while NE (448 ± 52 vs 320 ± 36 pg/ml) did not attain significance. The wide variability seen at time zero is most likely due to the small sample size and consequences of trauma induced by catheter placement in one patient. Presented as percent change increase over baseline values, a range of 68% to 178% was observed for E, and a range of -27.1% to 97.6% for NE (Figs. 1 and 2). The explanation for subject 5 decreasing below baseline for NE and having only a 32% increase in E over baseline may be made on the basis of significant anxiety and trauma experienced during venous catheter placement, resulting in elevated baseline values. When the data are represented as the mean percentage change over basal values (Fig. 3), significant differences are seen at 3 hr after theophylline therapy for E (p < 0.01) and NE (p < 0.05) compared with placebo. Epinephrine was increased 120% ± 25.3% with theophylline compared to 9.0% ± 8.67% with placebo, and NE increase was 48.0% ± 17.94% and 3.68% ± 8.0%, respectively. Significant differences between theophylline and placebo treatments were not seen at 1 or 2 hr for E and NE or at any of the time periods for DA (data not shown). Significant differences in catecholamine levels from baseline were not noted at any time after placebo administration.

Theophylline assay

Theophylline assay was performed by means of high-pressure liquid chromatography, with a sensitivity of less than 1 μg/ml, and the coefficient of variation was less than 2%.

Statistical analysis

Data were analyzed by a paired t test for mean differences between catecholamine levels after theophylline and placebo administration and for estimation of the significance of mean catecholamine differences over baseline. A Wilcoxon nonparametric test was also performed to eliminate the possibility of an abnormal distribution of data. In addition, linear regression analysis was utilized.

---

**TABLE I. Effect of theophylline on plasma catecholamine levels**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Mean theophylline level (μg/ml ± SEM)</th>
<th>DA Drug</th>
<th>Placebo</th>
<th>E Drug</th>
<th>Placebo</th>
<th>NE Drug</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>—</td>
<td>65 ± 10</td>
<td>102 ± 2</td>
<td>56 ± 18</td>
<td>69 ± 5</td>
<td>320 ± 36</td>
<td>278 ± 68</td>
</tr>
<tr>
<td>1</td>
<td>4.60 ± 0.67</td>
<td>46 ± 5</td>
<td>176 ± 18</td>
<td>56 ± 11</td>
<td>79 ± 8</td>
<td>285 ± 63</td>
<td>298 ± 68</td>
</tr>
<tr>
<td>2</td>
<td>5.80 ± 0.88</td>
<td>73 ± 8</td>
<td>53 ± 6</td>
<td>80 ± 18</td>
<td>74 ± 4</td>
<td>364 ± 64</td>
<td>254 ± 43</td>
</tr>
<tr>
<td>3</td>
<td>7.20 ± 0.48</td>
<td>77 ± 11</td>
<td>109 ± 47</td>
<td>105 ± 16</td>
<td>74 ± 6</td>
<td>448 ± 52</td>
<td>248 ± 35</td>
</tr>
</tbody>
</table>

**RESULTS**

The use of the commercially available Cat-A-Kit in our laboratory resulted in a 5% variability between duplicate samples and controls provided in the kit. The mean (± SEM) plasma E, NE, and DA levels (pg/ml) after theophylline and placebo administration are depicted in Table I. The mean (± SEM) theophylline level (μg/ml) is also shown at 1, 2, and 3 hr after drug administration. E serum concentration was significantly elevated (p < 0.001) at 3 hr as compared with baseline (105 ± 16 vs 56 ± 18 pg/ml), while NE (448 ± 52 vs 320 ± 36 pg/ml) did not attain significance. The wide variability seen at time zero is most likely due to the small sample size and consequences of trauma induced by catheter placement in one patient. Presented as percent change increase over baseline values, a range of 68% to 178% was observed for E, and a range of -27.1% to 97.6% for NE (Figs. 1 and 2). The explanation for subject 5 decreasing below baseline for NE and having only a 32% increase in E over baseline may be made on the basis of significant anxiety and trauma experienced during venous catheter placement, resulting in elevated baseline values. When the data are represented as the mean percentage change over basal values (Fig. 3), significant differences are seen at 3 hr after theophylline therapy for E (p < 0.01) and NE (p < 0.05) compared with placebo. Epinephrine was increased 120% ± 25.3% with theophylline compared to 9.0% ± 8.67% with placebo, and NE increase was 48.0% ± 17.94% and 3.68% ± 8.0%, respectively. Significant differences between theophylline and placebo treatments were not seen at 1 or 2 hr for E and NE or at any of the time periods for DA (data not shown). Significant differences in catecholamine levels from baseline were not noted at any time after placebo administration. However, a significant correlation (p < 0.05) was found between the percentage increase above baseline for E (r = 0.58) and NE (r = 0.66) and the serum theophylline level (Fig. 4).

Clinically, there were no significant increases in mean arterial blood pressure, pulse rate, or respiratory
rate in the five subjects after theophylline compared with placebo therapy. No untoward effects were experienced after theophylline administration during the study period.

DISCUSSION

The exact mechanism of action for theophylline has not been fully elucidated. Although phosphodiesterase inhibition has been widely accepted, there exists evidence that the tissue concentrations achieved with recommended doses do not fully inhibit the degradation of cAMP through inhibition of phosphodiesterase. Consequently, other researchers have proposed additional mechanisms by which the pharmacologic actions of theophylline are mediated. As mentioned above, other proposed mechanisms of action suggested for theophylline include prostaglandin inhibition, adenosine receptor antagonism, and increased cellular uptake of calcium. In addition to these research findings, James and Strubelt have demonstrated that adrenal catecholamines may play an important part in the mediation of pharmacologic effects of theophylline.

This pilot study demonstrated that theophylline administered as a single oral dose in clinically relevant concentrations can significantly increase the plasma catecholamine E and NE, but not DA. E was elevated to a greater degree over basal levels than was NE (120% compared to 48%), suggesting increased adrenal medullary secretion rather than generalized undifferentiated sympathetic stimulation. These findings are similar to those reported by Robertson et al. using caffeine. Previous studies had suggested that caffeine was one of the most potent stimuli of adrenomedullary secretion, but no data had been published with the effects of theophylline. Recently, however, Vestal et al. showed significant increases in plasma E and NE after intravenous aminophylline infusions that were related to the serum theophylline level. Thus E and NE levels were 45 ± 10 and 202 ± 18 pg/ml (mean ± SEM), respectively, with a theophylline level of 4.4 ± 0.3 μg/ml compared with 114 ± 28 and 308 ± 36 pg/ml when the theophylline level was 20.5 ± 1.4 μg/ml. Our data are similar to those of Vestal et al., but our study design differs in several aspects. The subject population was small, since we designed only a pilot study. We utilized a noncommercial tablet-containing capsule, which may have resulted in slow theophylline absorption and relatively low serum levels. Theophylline levels were still increasing at 3 hr and never reached therapeutic levels (10 to 20 μg/ml), as might be expected in a single-dose study. This may explain the gradual increase in E and NE, absence of clear dose-response correlation, and the lack of increase in cardiovascular parameters such as heart rate and mean arterial pressure observed in the previous study. We encountered considerable interpatient variability be-
between baseline and placebo- and theophylline-induced responses. For example, baseline values were considerably greater in one subject (No. 5), who required multiple venipuncture to properly insert the angiocatheter utilized. Differences in rates of theophylline absorption could have also contributed to the variability. These factors and the rather small number of subjects may have masked significant changes in E and NE responses to theophylline at 1 and 2 hr after drug administration. Nonetheless, the trend in these observations suggests that theophylline could exert pharmacologic effects through the release of adrenomedullary catecholamines. Although the plasma catecholamine levels found in this study are relatively low compared with those reported with currently utilized beta-2 adrenergic drugs, pharmacologic effects on cardiovascular and metabolic systems are thought to be due to the catecholamine increases with methylxanthines. This suggests that bronchial smooth muscle relaxation could be mediated or enhanced by catecholamines even at these concentrations, perhaps acting in an additive or synergistic mode with theophylline. Results from several animal models suggest the importance of the observation that the reduction in cholinergic-induced bronchoconstriction in guinea pigs after aminophylline infusion was markedly diminished after bilateral adrenalectomy. In addition, similar attenuation of the theophylline effect was seen after a beta-adrenergic antagonist was administered. Furthermore, adrenal medullectomy combined with reserpine therapy completely abolished theophylline- and caffeine-induced increases in blood glucose levels in anesthetized rats. Such experimental results imply that the release of catecholamines by theophylline may contribute to its pharmacologic action.

Further studies are indicated to determine more clearly the role of catecholamines in the therapeutic effect of theophylline. The effects of multiple theophylline dosing at therapeutic concentrations, continuous infusion of theophylline on adrenal secretion of E and NE, and comparison of plasma E and NE concentrations achieved with theophylline and beta-receptor agonists would be of considerable clinical interest. It is likely that some of the pharmacologic side effects observed with theophylline such as increase in heart rate, blood pressure, and tremor are the result of increased plasma catecholamines, as noted by Vestal et al. If these responses diminish with continued theophylline use, this may be explained on the basis of tachyphylaxis to beta-adrenergic stimulation. These initial observations are provocative in suggesting that theophylline-induced release of adrenal catecholamines may be an additional potential mechanism of action. Whether this is an important mechanism in the development of side effects or in asthma therapy remains to be determined.

REFERENCES
5. Horrobin DF, Manku JS, Franks D, Hamel P: Methyl xanthine phosphodiesterase inhibitors behave as prostaglandin an-


