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The Effects of L-Dihydroxyphenylalanine on Alertness and Mood in α-Methyl-Para-Tyrosine-Treated Healthy Humans

Further Evidence for the Role of Catecholamines in Arousal and Anxiety

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Treatment with α-methyl-para-tyrosine (AMPT), a catecholamine synthesis inhibitor, has been shown to produce pronounced increases in sleepiness and mild increases in negative mood and anxiety when administered to healthy male adults. The present study was conducted to ascertain whether these effects of AMPT are secondary to decreases in brain catecholamines or whether they represent nonspecific drug effects. Forty-one healthy males were randomized to one of four treatment groups. (1) Treatment with AMPT alone (AMPT/placebo); (2) treatment with AMPT plus L-dopa/carbidopa (AMPT plus L-dopa/carbidopa); (3) treatment with L-dopa/carbidopa alone (placebo plus L-dopa/carbidopa); or (4) treatment with placebo alone (placebo plus placebo). Repeated measures of alertness, mood, and anxiety were obtained over a three-day period of drug treatment and following drug discontinuation. As before, AMPT treatment led to increased sleepiness. In addition, AMPT treatment led to decreased calmness, increased tension and anger, and a trend for increased depression. Replacement of catecholamine stores with L-dopa reversed the effects of AMPT and was associated with a more rapid recovery from AMPT's effects. These findings indicate that AMPT's effects on alertness and anxiety are catecholamine-specific. Further, they provide additional evidence that catecholamines are involved in the regulation of normal states of arousal, and they are consistent with the view that brain catecholaminergic dysregulation is involved in pathological anxiety states. ***[Neuropsychopharmacology 13:41–52, 1995]***

KEY WORDS: *Catecholamines; Arousal; Mood; Anxiety; AMPT; L-dopa; Carbidopa*

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A large body of clinical and pharmacological data indicates that CNS catecholamines are involved in the modulation of alertness, mood, and anxiety (Betts 1981; Bunney and Davis 1965; Charney et al. 1984; Greenspan et al. 1969; Nicholson and Pascoe 1990; Redmond 1979). For instance, manipulations of CNS catecholamines that lead to increased catecholaminergic neurotransmission, such as d-amphetamine administration, lead to acute increases in alertness and improved mood (Newhouse et al. 1989). Conversely, drugs that diminish brain catecholamine neurotransmission (e.g., reserpine, methyldopa) can lead to depressed mood and diminished arousal.

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The role of catecholamines in anxiety is more complex, but in general, drugs that increase the synaptic availability of catecholamines, particularly norepinephrine (e.g., yohimbine), can lead to increases in anxiety (Charney et al. 1984; Nutt et al. 1990), while drugs that diminish catecholaminergic neurotransmission (e.g., clonidine) have anxiolytic effects (Charney and Heninger 1986). The view that catecholamines are involved in the regulation of mood and anxiety has been bolstered by studies in clinical populations of patients with mood and anxiety disorders, who have a therapeutic response to a number of tricyclic antidepressants (presumably due, in part, to actions on catecholaminergic substrates). Further, studies in patients with panic disorder have demonstrated that these patients are highly sensitive to the anxiogenic effects of noradrenergic-specific drugs (Charney and Heninger 1986), supporting the notion that abnormally high responsivity of brain noradrenergic systems is involved in the pathogenesis of panic disorder.

In a previous study (McCann et al. 1993) we investigated the role of CNS catecholamines in modulating alertness, mood, and anxiety in healthy adult males using α-methyl-para-tyrosine (AMPT), a tyrosine hydroxylase inhibitor. AMPT selectively inhibits the synthesis of catecholamines by blocking the rate limiting step in the catecholamine synthesis pathway (Nagatsu et al. 1964; Spector et al. 1965), and at adequate doses, it is associated with a 68% to 77% depletion in cerebrospinal fluid catecholamines (Brodie et al. 1971). Healthy male volunteers treated with AMPT developed significant decreases in alertness, as well as mild increases in depression and anxiety. Further, volunteers treated with AMPT and deprived of sleep for 40.5 hours developed profound increases in sleepiness as well as significant increases in depression and anxiety. These results provided further evidence for the role of catecholamines in alertness and mood but left open the possibility that AMPT's effects might be nonspecific.

The present study was designed to determine whether changes in alertness, mood, and anxiety previously observed in normal AMPT-treated humans were secondary to changes in catecholamines or whether they might have represented nonspecific effects of the drug. In particular, we sought to determine whether replacement of catecholamine stores with L-dopa/carbidopa [a combination of L-dihydroxy-phenyl-alanine (L-dopa), the endogenous precursor to catecholamines, plus L-α-hydrazino-α-methyl-β-(3,4-dihydroxybenzene) propanoic acid monohydrate (carbidopa), a peripheral decarboxylase inhibitor that increases the concentration of L-dopa available for entry into the CNS] would reverse the effects of AMPT in healthy human subjects. To accomplish this aim, subjects were randomized to one of four treatment groups: (1) treatment with AMPT alone (AMPT plus placebo); (2) treatment with AMPT plus L-dopa/carbidopa; (3) treatment with L-dopa/carbidopa alone (placebo plus L-dopa/carbidopa); or (4) treatment with placebo alone (placebo plus placebo). The effects of these treatments on alertness and mood were obtained by utilizing a visual analogue scale designed to assess global vigor and affect (Monk 1989), the Profile of Mood States (POMS, McNair et al. 1971) and multiple sleep latency tests (MSLTs, Carskaddon and Dement 1982).

METHODS

Subjects

Forty-one healthy nonsmoking male volunteers between the ages of 21 and 39 (mean age 24.5) were recruited through advertisements on local college campuses. After providing informed consent, subjects were screened using a self-rated questionnaire for depression and anxiety, a physical examination, a clinical psychiatric interview by a psychiatrist, an EKG, and blood and urine tests (including a SMAC-20, a CBC with differential, thyroid function tests, a hepatitis screen, HIV testing, a routine urinalysis, and a screen for drugs of abuse). Exclusion criteria included past or present major medical or psychiatric illness, a positive urine drug screen, a positive HIV or hepatitis test, an abnormal EKG, or a score of 6 or more (out of a possible 12) on either the anxiety or depression scale of the self-rated questionnaire. Inclusion criteria included normal sleeping patterns (6–8 hours of sleep between the hours of 10:00 P.M. and 10:00 A.M.), little to moderate caffeine and alcohol use (less than three cups of coffee or caffeinated soda per day and less than a six-pack of beer or the equivalent per week). Following completion of screening procedures, subjects were randomly assigned to one of the four treatment groups.

Procedure

Subjects arrived at the laboratory in groups of three or four at 6:00 P.M., two nights prior to the initiation of drug treatment (see Figure 1 for study timeline). At that time subjects provided a second urine sample for screening of drugs of abuse and were equipped with ambulatory polysomnographic scalp electrodes using the international 10–20 system of electrode placement. Electrodes were placed at C3, C4, A1, A2, O1, O2, L-EOG, R-EOG, FP2, and submandibularly. In addition, two chest leads were placed for ambulatory EKG monitoring. All leads were connected to an eight-channel Oxford Medilog ambulatory cassette recorder for continuous on-line recording. During the first evening in the laboratory subjects completed two training blocks on a computerized performance assessment battery (Walter Reed Performance Assessment Battery,

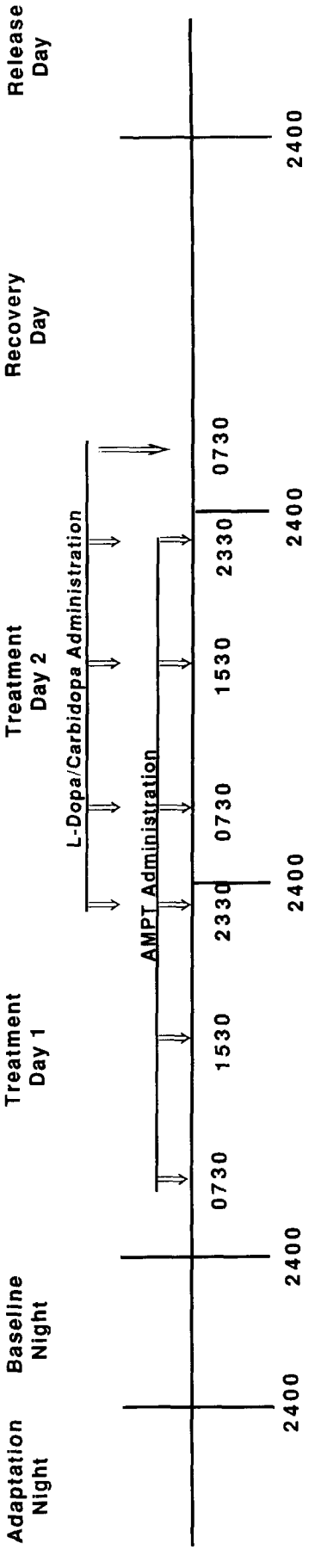

Figure 1. Generalized timeline for study procedures. Arrows indicate times of drug administration.

WRAIR PAB, Thorne et al. 1985). The particular version on the WRAIR PAB used contains a number of cognitive tests, as well as the Profile of Mood States and the Stanford Sleepiness Scale (Hoddes et al. 1973). Well-trained subjects typically take 20 minutes to complete the PAB.

In addition to PAB training and polysomnographic monitoring, during the first evening in the laboratory, an intravenous catheter was placed for serial blood draws. A total of three blood draws (10 cc/draw) was performed over a 1-hour period prior to removal of the intravenous catheter. Blood samples were immediately placed on ice and subsequently spun at 3600 rpm for 10 minutes. Serum samples were placed in a −70°C freezer for subsequent neuroendocrine analyses.

Following the second PAB training session at 11:30 P.M. subjects went to sleep (Adaptation Sleep) and were awakened at 7:00 A.M. the following morning when they completed a third training PAB. They left the laboratory until 6:00 P.M. that evening, when polysomnographic leads were replaced and three additional PAB training blocks were completed prior to sleep at 11:30 P.M. (Baseline Sleep). As previously, subjects were awakened at 7:00 A.M. the following morning, when drug treatment was initiated (Treatment Day 1).

Drug Treatment

As described, subjects were randomized to one of four drug treatment groups: (1) treatment with AMPT alone (AMPT plus placebo); (2) treatment with AMPT plus L-dopa/carbidopa; (3) treatment with L-dopa/carbidopa alone (placebo plus L-dopa/carbidopa); (4) treatment with placebo (placebo/placebo). AMPT was administered at a dose of 1 g three times daily (7:30 A.M., 2:30 P.M., and 11:30 P.M.) for 2 days (Treatment Days 1 and 2). This dose of AMPT was utilized because it has been shown to deplete effectively CSF catecholamines by 66% to 77% after 24 hours of treatment (Brodie et al. 1971). L-dopa/carbidopa was administered at a dose of 100 mg/25 mg three times a day (7:30 A.M., 2:30 P.M., and 11:30 P.M.), starting at 11:30 P.M. on Treatment Day 1 and stopping at 7:30 A.M. on Recovery Day (a total of 5 doses). This dose of L-dopa/carbidopa was chosen because it is a common dose for patients with Parkinson's Disease (i.e., patients thought to have at least an 80% depletion of dopamine in the substantia nigra (Forno and Alford 1987; Hornykiewicz 1975; Koller 1992), and in those patients it often effectively treats their symptoms. L-dopa/carbidopa administration was not started at the same time as AMPT administration because the purpose of the study was to determine whether L-dopa/carbidopa reverses the effects of AMPT. As might be anticipated by the different mechanisms of action of the two drugs, the effects of AMPT do not become apparent until several doses have been administered (subjects in the previous study began to experience sleepiness during the P.M. of Treatment Day 1), while the effects of L-dopa often appear within 30 minutes after administration. Hence L-dopa was started at 11:30 P.M. on treatment day 1, at the time of the third AMPT dose.

Measures of Alertness and Mood

Over the 3-day study period (i.e., Treatment Day, 1, Treatment Day 2, and Recovery Day) subjects completed a total of 24 POMS (approximately every 2 hours while awake) and 12 visual analogue scales. As an objective measure of physiological sleepiness, subjects took 12 sleep latency tests over the 3-day study period.

Serum Prolactin Measures

Serum prolactin concentrations were used as an indicator of AMPT efficacy. Specifically, since dopamine is known to inhibit prolactin secretion, depletion of CNS dopamine levels with AMPT should lead to a rise in serum prolactin. Conversely, elevated CNS dopamine levels (following administration of L-dopa/carbidopa) should lead to decreases in serum prolactin levels. Further, if the dose of L-dopa/carbidopa utilized in this study effectively replenishes CNS dopamine concentrations, combined AMPT plus L-dopa/carbidopa treatment should be associated with normal serum prolactin concentrations.

Data Analyses

Repeated measures analyses of covariance (ANCOVA) were used to evaluate the effects of AMPT, L-dopa/carbidopa, and the coadministration of AMPT plus L-dopa/carbidopa on measures of alertness and mood. Test scores prior to drug administration were used as covariates for all analyses. When significant main effects of drug or time by drug interactions were found, simple analyses of variance (ANOVA) were conducted within each individual time point. If simple main effects were observed at a particular time point on Treatment Days or Recovery Day, between-group differences were examined to identify significant relationships pertinent to the study's hypotheses. Planned comparisons were used on Treatment Day 1 (when the only active drug administered prior to 10:30 P.M. was AMPT) to examine the differences between placebo and AMPT on measures of alertness and mood. Post hoc Duncan tests were used to examine the effects of L-dopa/carbidopa administration on alertness and mood (Treatment Day 2). Specific relationships tested were: potential reversal of the effects of AMPT by L-dopa/carbidopa (AMPT + placebo versus AMPT plus L-dopa/carbidopa) and the effects of L-dopa/carbidopa alone (placebo + placebo

Table 1. Main Effects, Interactions, Comparisons, and Post Hoc Tests

Measure	Drug Effect	Time Effect	Drug × Time Effect
Alertness and fatigue	df = 3,34	df = 13,455	df = 39,455
VAS alertness	$F = 4.78^a$	$F = 3.85^b$	$F = 1.65^b$
VAS sleepiness	$F = 5.35^a$	$F = 2.91^b$	$F = 1.96^b$
VAS weariness	$F = 3.11^c$	$F = 2.88^b$	$F = 1.94^b$
VAS effort	NS	$F = 2.24^a$	$F = 1.64^a$
MSLT	df = 3,36 $F = 8.34^b$	df = 15,555 $F = 7.72^b$	df = 45,555 $F = 1.43^c$
Mood	df = 3,35	df = 11,396	df = 33,396
VAS happiness	$F = 3.08^c$	$F = 3.30^b$	NS
VAS calmness	$F = 5.24^a$	NS	$F = 1.41^c$
POMS tension	NS	NS	$F = 1.90^a$
POMS anger	NS	$F = 1.82^c$	$F = 1.90^a$

[a] Significant effect, $p < .01$.
[b] Significant effect, $p < .001$.
[c] Approaching significant effect, $p < .05$.

vs. placebo + L-dopa/carbidopa) on alertness and mood.

An ANOVA was used to compare the effects of the various drug treatments on serum prolactin concentration. The means of three serum prolactin concentrations obtained over a 1-hour period prior to drug treatment were compared to the means of three serum prolactin concentrations obtained over a 1-hour period on the afternoon of Treatment Day 2. Post hoc Duncan tests were then performed to determine which of the four treatment groups differed significantly. All statistical analyses were performed utilizing SPSS-X.

RESULTS

Results from the overall repeated measures ANCOVA for the Visual Analog Scales (VAS) and the MSLT are presented in Table 1. Significant contrasts (planned comparisons and Duncan post hoc tests) are presented in Table 2 and in the text. VAS and MSLT data met criteria for homogeneity of variance, whereas POMS data did not. Square-root tranformations were performed on the POMS subscales to normalize their distributions. Because of the large number of comparisons by ANCOVA, results from these comparisons were considered significant at a level of $p < .01$, and only significant or near significant results pertinent to the discussion are reported. Results obtained from planned comparison or Duncan contrasts were considered significant at a level of $p < .05$.

Measures of Alertness and Fatigue

Alertness and sleepiness, as measured by the VAS and MSLT, were significantly affected by AMPT administration and by the coadministration of AMPT and L-dopa/carbidopa. Overall, AMPT decreased alertness and increased fatigue, weariness, effort, and sleepiness (both by self-report and objective measures).

Alertness. (Measured using the VAS "Alertness" scale.) Significant Drug, Time, and Drug × Time effects were observed on alertness [$F(3,34) = 4.78$, $p < .01$, $F(13,455) = 3.85$, $p < .001$, $F(39,455) = 1.65$, $p < .01$, respectively]. As shown in Figure 2, AMPT administration decreased alertness, and L-dopa/carbidopa reversed this effect. Furthermore, the time course for decreases in alertness corresponds well with the expected time course of AMPT-induced catecholamine decreases in the CNS (Rech 1966). Planned comparisons revealed that at each time point on Treatment Day 1 (before L-dopa/carbidopa administration was initiated), subjects receiving AMPT reported significant decrements in alertness compared to subjects receiving placebo (p's $< .05$). L-dopa/carbidopa administration slowly reversed the effects of AMPT on alertness,

Table 2. Planned Comparisons[a] and Post Hoc Duncan Contrasts[b]

	Treatment Day 1				Treatment Day 2			Recovery Day			
	0800	1200	1600	2000	0800	1200	1600	0800	1200	1600	2000
Alertness		1	1	1	1	2, 5	2, 5	2, 4, 5	2, 3, 4	2, 3, 4	
Sleepiness				1	1		2, 3, 4, 5	2, 4, 5	2, 4	2, 3, 4	
Weariness				1	1	2, 5	2, 4	2, 4	2, 3, 4		
Effort			1	1	5, 6	2, 5	2, 4, 6				
MSLT		1	1	1	2, 4, 5	2, 4	2, 4, 5, 6		2, 4	2	
Calmness							2, 3	2, 3, 7, 6	2, 3		
Tension						2, 4		2, 3			
Anger								2, 6			

[a] Planned comparisons ($p < .05$): 1 = AMPT vs. placebo.
[b] Duncan post hoc tests ($p < .05$): 2 = AMPT + placebo vs. placebo + placebo; 3 = AMPT + placebo vs. AMPT + L-dopa/carbidopa; 4 = AMPT + placebo vs. placebo + L-dopa/carbidopa; 5 = AMPT + L-dopa/carbidopa vs. placebo + placebo; 6 = AMPT + L-dopa/carbidopa vs. placebo + L-dopa/carbidopa; 7 = placebo + L-dopa/carbidopa vs. placebo + placebo.

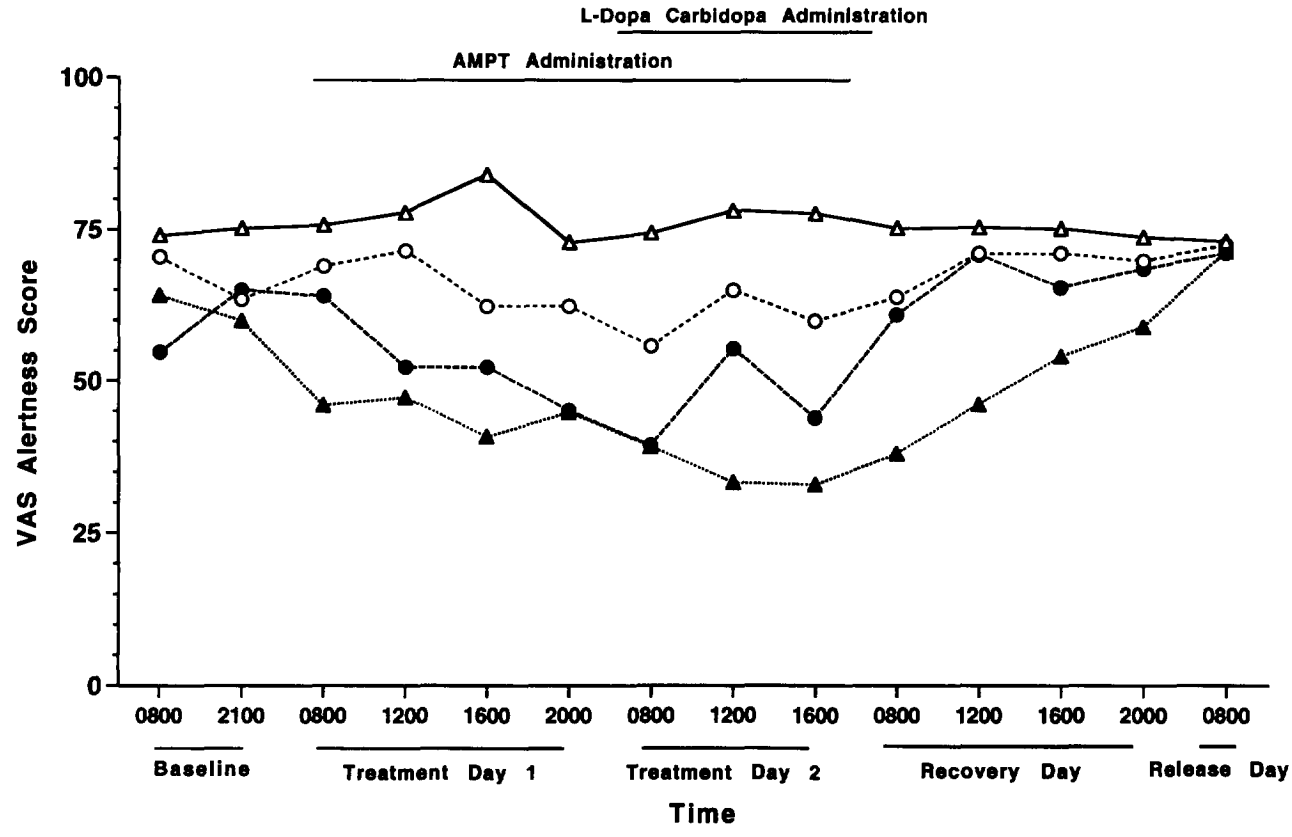

Figure 2. Self-rated alertness using a visual analogue scale. Main effects, interactions, contrasts, and post hoc comparisons are provided in Table 1 and are described in the text. (*Open triangles*: placebo + placebo; *filled triangles*: AMPT + placebo; *open circles*: placebo + L-dopa/carbidopa; *filled circles*: AMPT + L-dopa/carbidopa.)

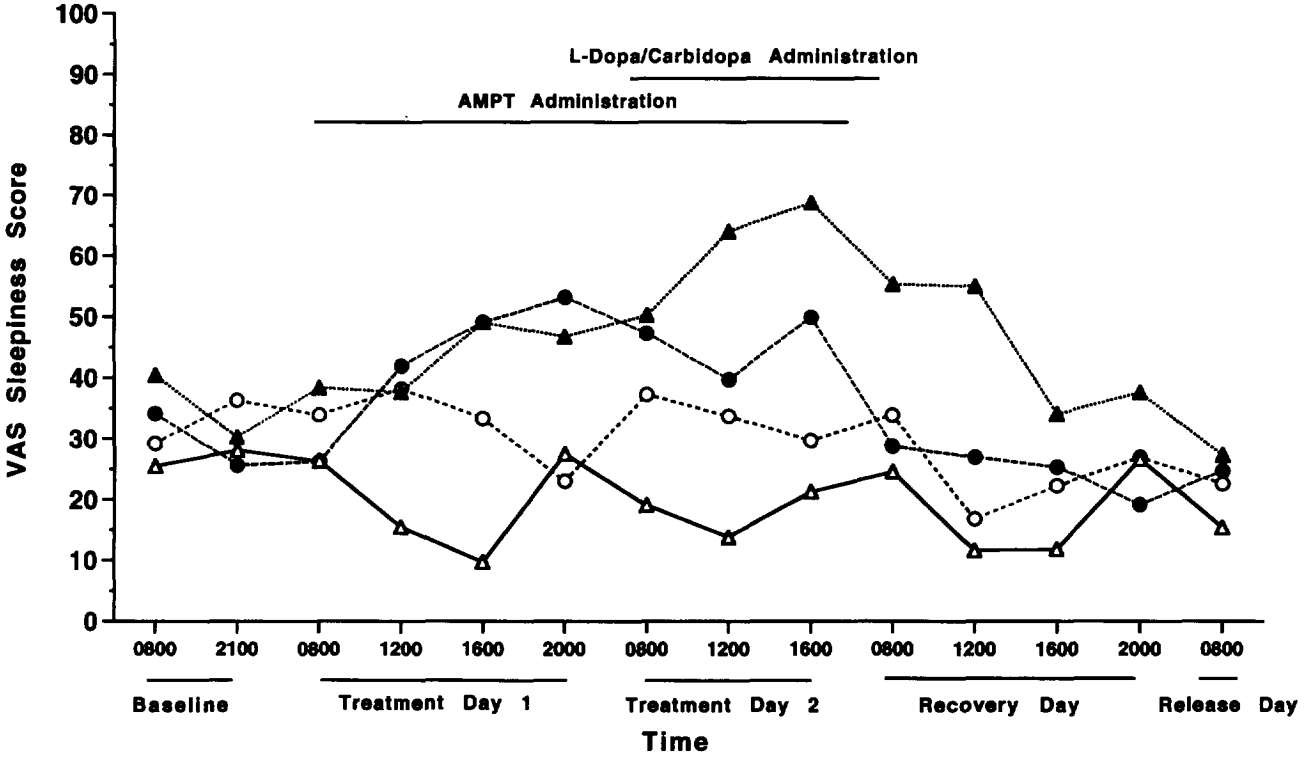

Figure 3. Self-rated sleepiness using a visual analog scale. Main effects, interactions, contrasts, and post hoc comparisons are provided in Table 1 and are described in the text. (*Open triangles*: placebo + placebo; *filled triangles*: AMPT + placebo; *open circles*: placebo + L-dopa/carbidopa; *filled circles*: AMPT + L-dopa/carbidopa.)

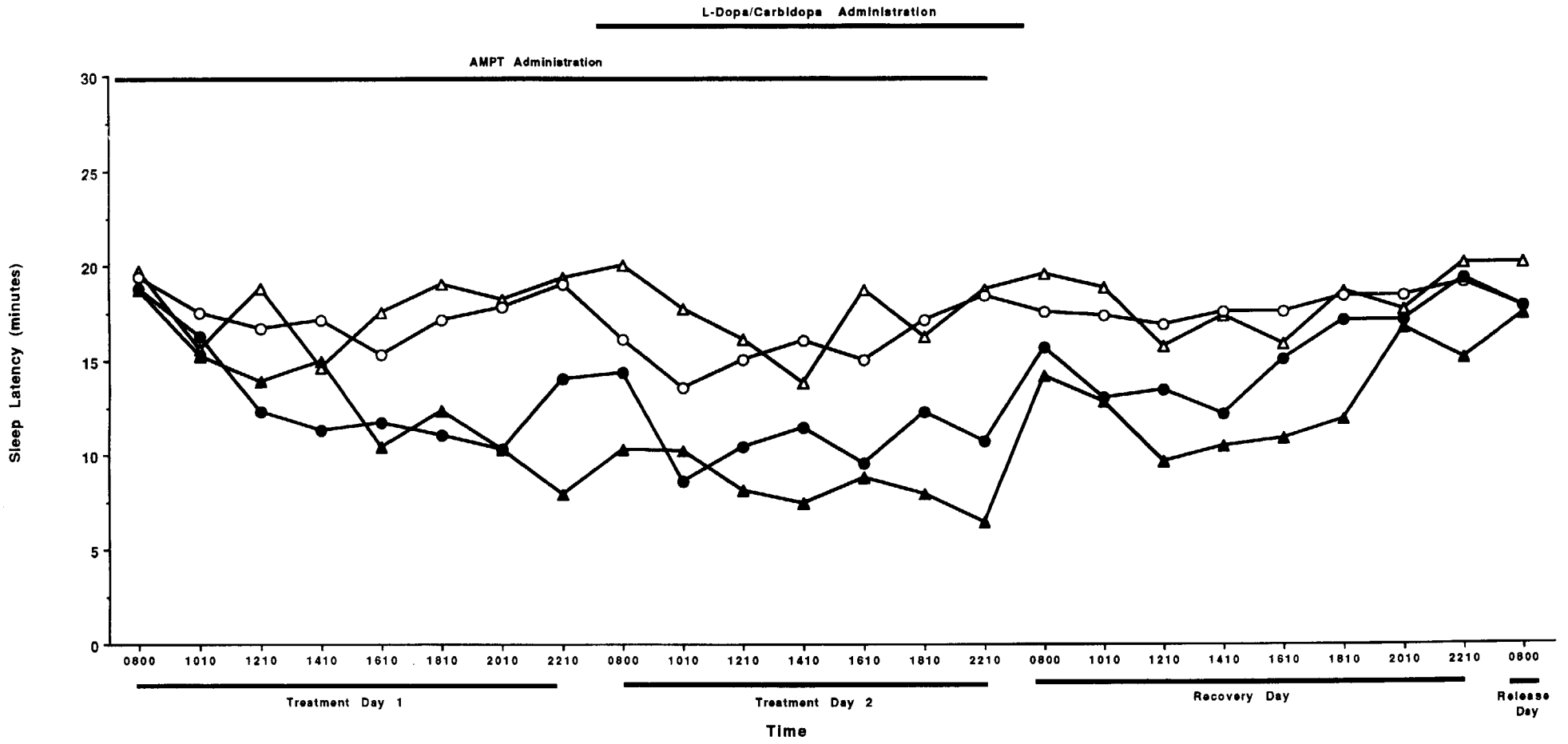

Figure 4. Number of minutes before sleep onset, as assessed by the Multiple Sleep Latency Test. Main effects, interactions, contrasts, and post hoc comparisons are provided in Table 1 and are described in the text. (*Open triangles*: placebo + placebo; *filled triangles*: AMPT + placebo; *open circles*: placebo + L-dopa/carbidopa; *filled circles*: AMPT + L-dopa/carbidopa.)

with measures of alertness beginning to increase by noon on Treatment Day 2 in the AMPT plus L-dopa/carbidopa group (after two doses of L-dopa/carbidopa) and was significantly greater than in the AMPT plus placebo group at 8:00 A.M. and noon on Recovery Day (p's < .05). L-dopa/carbidopa alone did not significantly affect alertness.

Sleepiness and Fatigue. (Measured using the VAS "Sleepiness," "Weariness," and "Effort" Scale and the POMS "Fatigue" scale.) Sleepiness was significantly affected by drug and time, with a significant drug × time interaction [$F(3,33) = 5.35$, $p < .01$, $F(13,442 = 2.91$, $p < .01$, $F(39,442) = 1.96$, $p < .01$, respectively]. Specifically, on measures of weariness and effort, time and drug × time interactions were found [weariness, Time: $F(13,442) = 2.88$, $p < .01$, Drug × Time: $F(39,442) = 1.94$, $p < .01$; effort, Time: $F(13,455) = 2.24$, $p < .01$, Drug × Time: $F(39,455) = 1.64$, $p < .01$]. As would be expected, the effects of AMPT and L-dopa/carbidopa on measures of sleepiness and fatigue were the inverse of their effects on alertness. In particular, AMPT administration led to increased sleepiness, and L-dopa/carbidopa attenuated this increase. The effects of AMPT on sleepiness were first observed on Treatment Day 1 (prior to initiation of L-dopa/carbidopa replacement), when subjects receiving AMPT reported greater sleepiness at 4:00 and 10:00 P.M. (p's < .05). Measures of sleepiness in the L-dopa/carbidopa replacement group began to decrease during Treatment Day 2, while sleepiness measures in subjects who took AMPT alone increased (see Figure 3). By the end of Treatment Day 2, measures of sleepiness in the L-dopa/carbidopa replacement group were significantly less than those in the AMPT alone group. The effect of AMPT on sleepiness measures persisted until 4:00 P.M. on recovery day, when measures in the AMPT alone group were no longer significantly different from those in other groups (16½ hours after the last AMPT dose). Scores on the "effort" scale were consistent with those on the sleepiness scale, with all subjects who received AMPT reporting that greater "effort" was required to accomplish tasks. However, subjects who received L-dopa/carbidopa replacement were affected less than those who did not and recovered more quickly than subjects in the AMPT plus placebo group.

Sleep Latency. (An objective measure of sleepiness, using the Multiple Sleep Latency Test.) Changes in alertness and fatigue were also found using a physiological measure of sleepiness [Drug $F(3,34) = 4.78$; $p < .01$, Time $F(13,455) = 3.85$, $p < .01$, Drug × Time $F(39,455) = 1.65$, $p < .01$] (Figure 4). Specifically, AMPT administration led to significant decreases in sleep latency (reflecting increased physiological sleepiness). On treatment Day 1 (prior to initiation of L-dopa/carbidopa replacement), subjects receiving AMPT had shorter sleep latencies than those receiving placebo, with differences increasing over the course of the day (p's < .05). On Treatment Day 2 and through most of the recovery period, subjects receiving AMPT alone continued to exhibit the shortest sleep latencies (p's < .05). Subjects who received L-dopa/carbidopa replacement had less consistent decreases in sleep latency, with differences between the AMPT plus placebo and the AMPT plus L-dopa/carbidopa groups appearing at measurement points shortly after L-dopa/carbidopa administration (i.e., at 8:00 A.M. and 2:00 P.M. on Treatment Day 2), as well as during the early evening on Treatment Day 2 (when effects of AMPT in the AMPT plus placebo group would be expected to be maximal). As with subjective measures of sleepiness, AMPT's effects on sleep latency began to dissipate during Recovery Day, and by the close of the day, no differences were apparent between any of the four treatment groups.

Measures of Mood

Mood was not robustly affected by AMPT or by coadministration of AMPT and L-dopa/carbidopa. Nevertheless, the data indicate AMPT had negative effects on mood that were reversed by dopamine replacement.

Positive Mood. (Measured using the VAS "Happiness" and "Calmness" scales.) As illustrated in Figure 5, the most consistent change in positive mood was a slow decrease in calmness in subjects who received AMPT alone (AMPT plus placebo) with a moderate decrease in calmness in subjects receiving L-dopa/carbidopa alone (placebo plus L-dopa/carbidopa), reflected by a significant drug effect [$F(3,34) = 5.24$, $p < .01$]. During Treatment Day 1, no significant differences were observed between patients who received AMPT and those who received placebo. However, by noon on Treatment Day 2, subjects who continued to take AMPT alone reported feeling significantly less calm than did subjects receiving either placebo alone or AMPT plus L-dopa/carbidopa (p's < .05). AMPT's effects on calmness persisted through 8:00 A.M. on Recovery Day (p < .05). Calmness scores for subjects receiving L-dopa/carbidopa alone were consistently lower than subjects receiving either placebo alone or AMPT plus L-dopa/carbidopa; however, this difference reached statistical significance at only one time point on Treatment Day 2 (4:00 P.M.). Finally, there were marginal effects observed on the "Happiness" scale [Drug: $F(3,33) = 3.08$, $p < .04$; Time: $F(13,442) = 3.3$, $p < .01$]. As with calmness, the AMPT plus placebo group consistently reported the greatest change in happiness compared to subjects who received placebo alone, although this difference was not statistically significant at any particular time point. In contrast to what was observed on the "Calmness" scale, L-dopa/carbidopa treatment

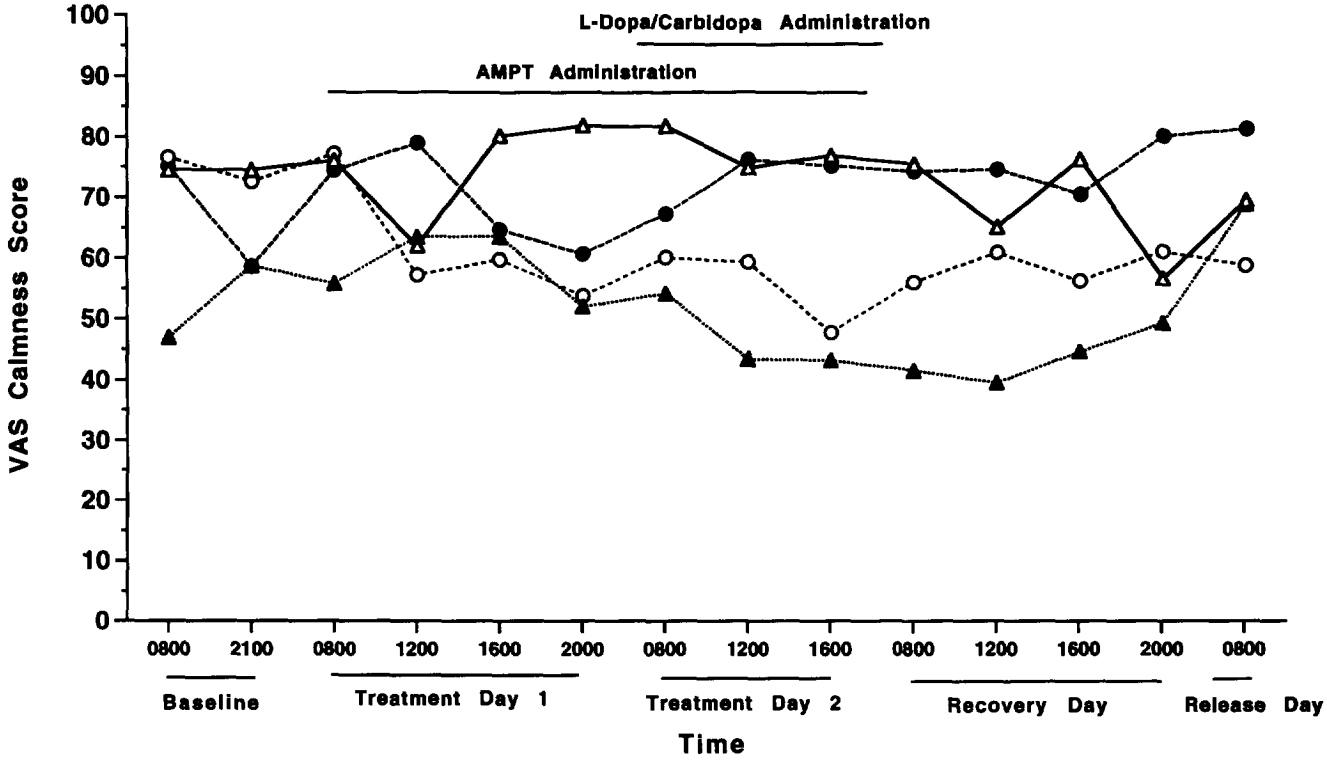

Figure 5 Self-rated calmness using a visual analogue scale. Main effects, interactions, contrasts, and post hoc comparisons are provided in Table 1 and are described in the text. (*Open triangles*: placebo + placebo; *filled triangles*: AMPT + placebo; *open circles*: placebo + L-dopa/carbidopa; *filled circles*: AMPT + L-dopa/carbidopa.)

alone did not result in a trend for decreased happiness, with subjects in that treatment group most closely approximating those within the placebo plus placebo group.

Negative Mood. (Measured by the VAS Sadness and Tension Scales and the POMS Sadness, Tension, and Anger Scales.) Significant drug × time effects were observed on the POMS Tension and Anger scales. In particular, subjects who received AMPT alone reported greater tension and anger than subjects who received placebo. Indeed, a subgroup of subjects who received AMPT alone consistently reported maximal ratings of tension, creating large variances in the group as a whole. As was noted with measures of alertness, L-dopa/carbidopa administration was found to reverse the anxiogenic effect of AMPT but did not lead to significant decreases in the anger-inducing effects of AMPT.

In addition to these significant effects, a nonsignificant trend was observed in subjects who received L-dopa/carbidopa alone. These subjects consistently reported higher measures of tension than subjects who received placebo alone (the inverse of what was observed on the calmness scale previously mentioned). Also, subjects who received AMPT alone or in combination with L-dopa/carbidopa tended to report increased sadness compared to the other two groups.

Physical Symptoms

In general, all drug treatments were well tolerated by study participants. In particular, there were no differences reported between subjects who received placebo and those who received active drug on measures of nausea or gastrointestinal discomfort (including diarrhea), the two most commonly reported side effects of L-dopa and AMPT respectively. Indeed, the only "side effect" that led two subjects to withdraw from the study prematurely was the development of panic attacks. Both of these subjects were later determined to have received AMPT. No data from these two subjects were included in the present analyses.

Serum Prolactin Measures

While there were no differences between treatment groups prior to drug treatment, all four groups differed by the afternoon of Treatment Day 2 [$F(1,3) = 40.01$, $p < .00001$; see Table 3]. As before, AMPT treatment alone led to a significant rise in serum prolactin. Subjects who received L-dopa/carbidopa replacement had significantly lower prolactin levels than those who received AMPT plus placebo, but they still had higher prolactin levels than those who received placebo alone and those who received L-dopa/carbidopa alone.

Table 3. Prolactin Concentrations in AMPT- and L-Dopa-Treated Subjects

	Pretreatment	Day 2 of Treatment
AMPT/placebo	20.17 (8.02)	58.04 (14.07)[a]
AMPT/L-dopa/carbidopa	15.4 (4.72)	43.81 (9.19)[a]
Placebo/L-dopa/carbidopa	16.0 (5.07)	16.04 (5.18)[a]
Placebo/placebo	19.94 (5.78)	25.62 (8.02)[a]

[a] Significantly different from all other treatment groups; $p < .001$. Values are group means (standard deviations.

DISCUSSION

The primary finding of this study is that the effects of AMPT on alertness and anxiety are reversed by treatment with L-dopa/carbidopa. This finding strongly suggests that the effects of AMPT are catecholamine specific, not due to nonspecific effects of AMPT. Further, the present data support the view that brain catecholamines are involved in the regulation of arousal and anxiety states.

In addition to the primary finding of the study, results from the present study indicate that in normal humans, decreases in CNS catecholamines can lead to increases in measures of anxiety. As alluded to in the Introduction, anxiety is typically thought to be associated with increases in catecholaminergic neurotransmission, and a large body of preclinical and clinical data support this notion (Charney and Heninger 1986; Redmond 1979; Nutt et al. 1990). However, in some instances, anxiety states have been observed during states of decreased arousal at times when catecholamine neurotransmission is diminished. For example. a growing body of literature indicates that a subpopulation of patients with panic disorder experience panic attacks during sleep (Hauri et al. 1989; Mellman and Uhde 1989, 1990; Uhde 1994). Polysomnographic techniques have been utilized to determine the electrophysiological characteristics of "sleep panic attacks," and in general they have shown that sleep panic occurs during or near the Stage 2-3 sleep transition (during a period of diminishing arousal and decreasing noradrenergic neuron activity). The observation that diminished catecholaminergic concentrations may be associated with anxiety is also consistent with the observation that some normal individuals have panic attacks when being treated with AMPT (McCann et al. 1991). The nature of AMPT-induced panic is not clear, however, and could possibly be secondary to postsynaptic receptor upregulation that may occur in the setting of diminished catecholamine neurotransmission.

As before (McCann et al. 1993), results from the present study indicate that in healthy individuals, AMPT does not induce significant increases in depression. This is in contrast to what has been observed in unmedicated patients with affective illness (Bunney et al. 1971) who responded to AMPT with significant increases in depression or decreases in manic symptoms. This observation, along with the observation that antidepressant medications do not improve mood in healthy individuals, suggests that catecholaminergic systems in depressed populations may be fundamentally different from those in healthy populations.

One aspect of the present data in particular deserves comment. As clearly shown in Figures 2–5, although L-dopa/carbidopa was found to reverse the effects of AMPT, they were not totally eradicated. While this could be taken to mean that some of AMPT's effects were noncatecholaminergic in nature, this is probably not the case. Instead, it is more likely that the dose of L-dopa/carbidopa utilized (100 mg L-dopa/25 mg carbidopa), as well as its short elimination half-life (1.5 hours), explain the subtotal nature of its effects. This low dose was utilized because it is known to exert behavioral effects in patients with early Parkinson's disease, yet in most patients it is not associated with significant nausea (the most problematic and common adverse effect of L-dopa treatment). Indeed, when the submaximal dose utilized is considered, the data may be even more convincing. Specifically, on measures of both alertness and calmness (Figures 2–4), following medication discontinuation, subjects who received AMPT alone have parallel recovery to those who received L-dopa/carbidopa replacement, and the time course of recovery parallels the time course of post-AMPT catecholamine repletion documented in brains of AMPT-treated animals (Rech et al. 1966). Pharmacokinetic differences between the two drugs utilized may also account for the jagged appearance of the sleep latency plots during treatment day 2 (Figure 5). Specifically, shortly after L-dopa administration, when peak pharmacological effects would be expected, subjects who received L-dopa have increases in sleep latency. These increases are not sustained, however, and with time (and diminished expected pharmacological effects), sleep latencies become similar to those of subjects in the AMPT plus placebo group. Finally, the notion that catecholamine stores were not totally replenished by the dose of L-dopa/carbidopa used is supported by neuroendocrine data, which indicates that although L-dopa/carbidopa administration led to significant decreases of prolactin in AMPT-treated subjects (reflecting increased CNS dopamine), they did not fall the lev-

els seen at baseline (or in placebo treated subjects).

It is recognized that ideally, full dose-response determinations would have been performed for both drugs studied. However, the number of subjects, the expense, and the time required for such studies in humans precluded this possibility. Future studies are needed to determine whether larger doses of L-dopa/carbidopa would be tolerated acutely, and if so, whether they more completely reverse the effects of AMPT. Given the finding that L-dopa/carbidopa itself may be anxiogenic, higher doses may be poorly tolerated either because of anxiety or nausea. Another potential limitation of the present findings is that behavioral rating data were based on subjects' self-reports. While it would have been preferable to also utilize data from observer-based ratings, the large number of personnel involved in each 6-day study run led to large interrater differences and to the decision to exclude these data from analyses. Interestingly, on measures of sleepiness, subjective self-ratings were found to be a better tool for distinguishing between extreme levels of sleepiness than objective measures (i.e., the MSLT), a finding also observed in our previous study (McCann et al. 1992).

When considering alternative explanations for the present experimental findings, it is important to note that manipulations of CNS catecholamine systems are also likely to influence other neurotransmitter systems (e.g., serotonin, GABA) as well as neuropeptides (e.g., CCK). The possibility that changes in alertness, mood, and anxiety demonstrated following AMPT (and/or L-dopa/carbidopa) are, in fact, indirect or downstream effects of catecholamine changes, remains.

In sum, the present results suggest that AMPT-induced decreases in alertness and calmness in normal humans are due to its effects on brain catecholamine systems. These findings further implicate catecholamines in normal arousal states, and support the notion that dysregulation of brain catecholamine systems may underlie some anxiety disorders.

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