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Chronic Hydrochlorothiazide and Verapamil Effects on Motor Activity in Hypertensive Baboons

JAYLAN S. TURKKAN,*[1] RICHARD P. ALLEN† AND ROBERT D. HIENZ*‡

**Department of Psychiatry and Behavioral Sciences, Division of Behavioral Biology*
†Sleep Disorders Center
‡Center for Hearing Sciences
The Johns Hopkins University School of Medicine, Baltimore, MD 21205

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TURKKAN, J. S., R. P. ALLEN AND R. D. HIENZ. *Chronic hydrochlorothiazide and verapamil effects on motor activity in hypertensive baboons.* PHARMACOL BIOCHEM BEHAV **41**(3) 567–572, 1992.—Spontaneous motor activity was measured in six baboons during chronic oral dosing with a diuretic (hydrochlorothiazide/triamterene), a calcium channel blocker (verapamil), and a combination of the two drugs. Piezoelectric monitors sensitive to movement were attached to leather collars and were worn continuously by the baboons throughout the protocol. Baboons were made hypertensive during a preexperimental period by either 1) chronic administration of deoxycorticosterone acetate (DOCA)-salt or 2) surgical renal artery stenosis. Total inactive periods/day increased over baseline levels during diuretic alone and increased further during diuretic + verapamil combined. The total number of inactive periods/day returned toward baseline levels in the subsequent conditions of verapamil alone and baseline recovery. Activity levels decreased during combination dosing mainly during morning hours (0700–1100 h). Overall changes in activity occurred in the second week of dosing; this time period was found earlier to maximally decrease blood pressure and to impair behavioral performances.

Thiazide diuretics Hydrochlorothiazide Calcium channel blocking agents Verapamil Motor behavior Renovascular hypertension DOCA-salt hypertension Blood pressure Antihypertensive agents Adverse side effects Nonhuman primates Baboons

FEW studies have directly examined the behavioral side effects of diuretics in the laboratory. A side effect of some concern during diuretic therapy has been potassium loss (hypokalemia), known to result in muscle weakness and lethargy. Despite frequent reports of sexual impairment and fatigue, however, direct central effects of the diuretics have not been experimentally demonstrated (18).

The calcium antagonists have been recently added to the armamentarium of hypotensive medications, and their behavioral effects are coming to be studied more extensively in the laboratory (4,8,9,26). We have reported adverse effects of the dihydropyridine calcium antagonist nifedipine on sensory discrimination and motor performances of both normotensive and renovascular hypertensive baboons during acute and chronic oral dosing (23,24). The nondihydropridine calcium antagonist verapamil is less potent with regard to chronotropic and smooth muscle contraction properties in comparison to nifedipine and other dihydropyridine calcium antagonists (20), and has produced fewer behavioral effects in direct comparisons with nifedipine in animal studies (7). Clinically, verapamil has been shown to enhance the hypotensive effect of diuretics in controlled studies with hypertensive patients (3). Side effects such as headaches and flushing have been occasionally reported in clinical evaluations during combined antihypertensive medications with calcium antagonists [e.g., (27)].

In open-field designs with rodents, some antihypertensive compounds have been found to increase activity [e.g., methyldopa (11)], while others have decreased open-field activity [e.g., propranolol (6)]. One of the advantages of studying general activity is that drug effects are not necessarily modulated by a task learning process (i.e., animals may overcome the effects of some drugs during well-learned behavioral tasks, a phenomenon known as "behavioral tolerance"). Continuous monitoring of activity also can provide an indirect index of sleep disturbance (1,2), often reported by patients during chronic antihypertensive dosing regimens (12,21).

This report examines the effects of an orally administered diuretic (hydrochlorothiazide) alone, verapamil alone, and

[1] Requests for reprints should be addressed to Jaylan S. Turkkan, Ph.D., Department of Psychiatry and Behavioral Sciences, Suite 3000, Behavioral Biology Research Center, The Johns Hopkins University School of Medicine, 5510 Nathan Shock Dr., Baltimore, MD 21224.

combined diuretic and verapamil on continuous spontaneous motor activity during chronic dosing. Drugs were studied in baboons whose blood pressures were preexperimentally elevated, yielding low-renin [deoxycorticosterone acetate (DOCA)-salt] and high-renin (renovascular) associated blood pressure elevations (14). Two baboon models of hypertension were employed to allow for the possible dissociation of the behavioral effects from the pressure-lowering effects of calcium antagonist and diuretic compounds. The effects of diuretics on motor activity may be attributable, for example, to lowered blood pressure in DOCA-salt subjects, but not in renovascular hypertensive subjects. Thus, a model was provided for each drug class that would be responsive to the hypotensive actions of the drugs.

METHOD

Subjects

Six adult male baboons (*Papio cynocephalus* and *Papio anubis*) served as subjects. Animals ranged in weight between 17–28 kg. Three baboons surgically prepared with unilateral renal artery stenosis (2-kidney, 1-clamp) were obtained from the Southwest Foundation for Biomedical Research [San Antonio, TX; see (15) for details of the surgical procedures] approximately $2\frac{1}{2}$ years prior to the current study, referred to as the "renovascular" baboons. The remaining three baboons were surgically intact and had their blood pressures preexperimentally elevated with dietary sodium and steroid administration (see below).

Apparatus and Environment

Animals were housed in individual living cages [(LWH) 48 × 35 × 56″) except during daily 90-min behavioral testing periods. Cages contained a seating bench and a spout for ad lib ingestion of tapwater. Two housing rooms each contained between 7–10 other individually caged baboons. An individual baboon's field of view included at least one other baboon at all times. The light:dark cycle of the housing rooms was 12 L:12 D; lights were on at 0600 h.

Animals wore a noninvasive, 1-oz activity-monitoring device with no external wires [LWH = 5.5 × 3.3 × 1.5 cm; Individual Monitoring Systems, Inc., Baltimore MD] that was slightly modified from one developed at NIH (5). The modification adjusted the response frequency to be maximum for 0.5–10 Hz, the range noted to be most significant for body movements in humans (17). The monitor was inserted into a padded metal container mounted on the outer surface of a soft leather collar. The collar did not interfere with eating, was well-tolerated, and allowed animals' access to all body parts. Movement by an animal triggered a piezoelectric sensor inside the monitoring device. The monitor recorded activity density by counting the number of 0.44-s intervals during which any significant movement occurred with acceleration greater than 0.1 g. Activity density is the proportion of these intervals during a 30-min period. For intense movement such as running or rapidly jumping up and down, the movement density approaches 100% (proportion × 100) and for inactive periods such as during sleep the activity density is nearly 0%.

The activity density for each successive 30-min period was recorded by the unit for up to 21 days; at the end of the recording period, the data were transferred from the monitor to a computer for analyses. Within-subject reliability of activity measurement across days was determined by use of odd-even day (i.e., test–retest) correlations [e.g., (19)] during a 260-day prestudy evaluation period of the monitoring system in two baboon subjects. Correlations of odd even day activity density were significant at $p < 0.01$ (Pearson $r = 0.513$ and 0.525 for each of two baboons).

Procedures

Salt diet and steroid administration. The three surgically intact baboons were administered salt as 5% hypertonic saline in flavored tap water for ingestion (15 g NaCl/day) in combination with DOCA (2.5 mg IM once/2 days) for 8 weeks prior to and during the study [see (22,25) for details]. Systolic/diastolic blood pressures were stable and chronically elevated by 14/5 mmHg over resting levels by the start of the current protocol (25).

Antihypertensive pharmacotherapy. Drugs studied were the calcium antagonist verapamil chloride (3.2 mg/kg/day) and the diuretic combination of hydrochlorothiazide (25 mg/day) and triamterene (50 mg/day) (Sigma Pharmaceuticals, St. Louis, MO). The potassium-sparing diuretic triamterene was added to hydrochlorothiazide to avoid life-threatening levels of hypokalemia. Each drug type was weighed in powdered form and inserted into a separate piece of fruit as vehicle. A double-dummy procedure was used such that two pieces of fruit were always cut open and administered regardless of whether single or combined drugs were scheduled for dosing.

Consecutive phases of drug administration consisted of: 1) a 14-day baseline (daily vehicle alone) followed by a 14-day active dosing period with daily diuretic administered alone; 2) diuretic and verapamil administered together during a 21-day daily dosing period ("combination drug period"); 3) a 14-day period of verapamil administered alone daily; and, 4) a 14-day vehicle-alone condition ("postdrug") at the end of the protocol.

During this 11-week study, animals were tested daily on a visual conditional discrimination task during 90-min experimental sessions conducted in a separate testing chamber. Color discrimination performance and response reaction time data measured during the sessions have been reported elsewhere (25). Drug administration/testing session hours were individualized for each animal, and ranged across animals from 0800–1230 h, Monday–Friday. Testing sessions were distributed equally throughout the day for DOCA-salt and renovascular baboons; the drug dosing and testing schedule of each animal was kept constant throughout the study. Verapamil was administered twice daily on weekdays at a 3-h interval and diuretic was administered once daily. During baseline and baseline recovery conditions, vehicle alone was administered twice daily. On weekends, animals were administered drug at 0900 and 1500 h.

Data analysis. A "day" was defined as a 24-h period beginning at 0300 h. For comparison of the treatment conditions, data were analyzed for the second week (5 days) on each treatment. Three measures of activity were summarized for data analysis: 1) average activity density/day (activity density/30 min as an average for 24 h); 2) total inactive periods/day (total number of 30-min periods/24 h containing zero activity); 3) consecutive inactive periods/day (total number of consecutive 30-min periods of zero activity/24 h). Finally, sleep fragmentation was indexed by the number of inactivity interruptions in a 7-h late-night period (2000–0300 h).

Hypothesis testing employed repeated-measures analysis of variance (ANOVA) for group (renovascular vs. DOCA-salt), condition (based on the second week of each drug condition), time of week (weekday vs. weekend), and other within-day

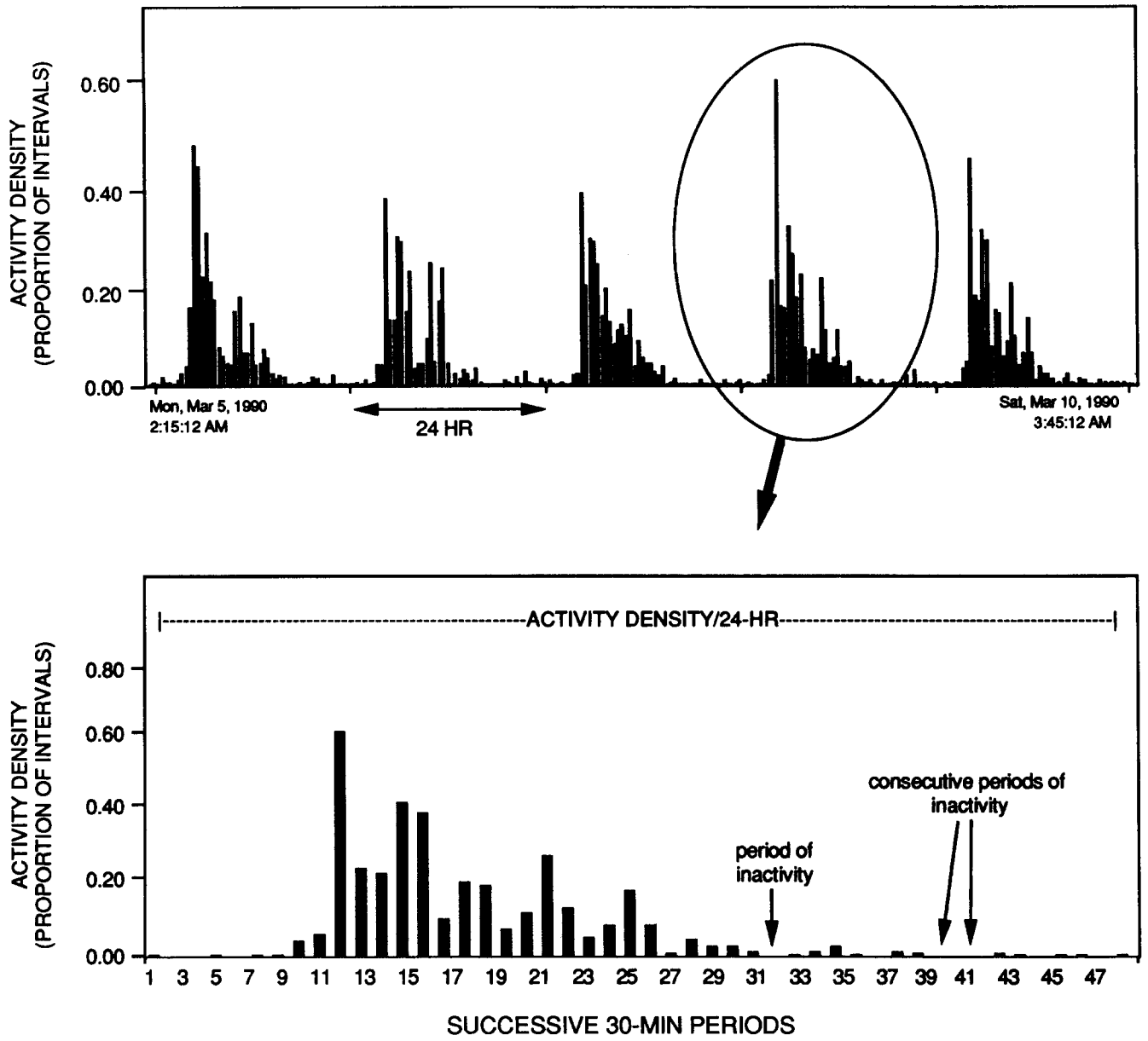

FIG. 1. Baseline (last 5 days) 24-h circadian patterns of activity density in 30-min time bins for one baboon. The lower panel illustrates the indices used for assessment of activity and inactivity: Denoted with arrows are a 30-min period of inactivity and two consecutive 30-min periods of inactivity. Total 24-h activity density was assessed by averaging all activity densities over 24 h (beginning at 0300 h).

within-group activity measures as described above. Probability levels less than or equal to 0.05 were considered significant. The more conservative Huynh-Feldt probability levels were used to control for violations of sphericity due to repeated measures, which may result in inflated ANOVA F ratios. Posthoc comparisons of means were conducted with Fisher's LSD test.

RESULTS

Baseline Conditions

During the baseline (vehicle only) condition, activity density/day was highly variable across all baboons and averaged 0.166 (±SEM 0.034) over all 30-min intervals. Total number of inactive periods/day averaged 7.05(±0.96) (a total of approximately 3½ h), and number of consecutive inactive periods/day averaged 1.08(±0.23) (about ½ h). Hypertension groups were not significantly different with respect to any activity measure during baseline (all $p > 0.30$). Figure 1 shows continuous activity of a single baboon across the last 5 weekdays (Monday–Friday) of baseline, with each of the three activity indexes marked for illustrative purposes in the lower panel. The majority of this animal's active periods occurred in the morning and early afternoons, a time period in which vehicle (fruit) was administered, the experimental session occurred, and the animal was then fed its full daily ration

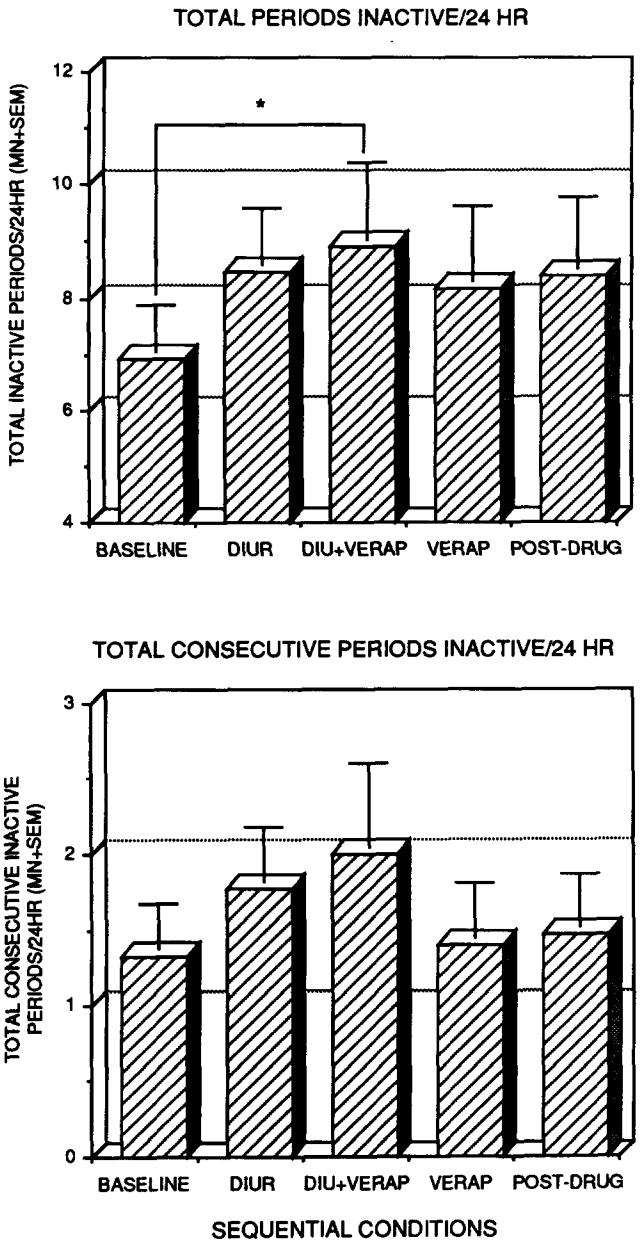

FIG. 2. Period of inactivity/24 h as a function of sequential experimental conditions. Total number of 30-min periods with inactivity in a 24-h period (upper panel) and total number of consecutive periods with inactivity in a 24-h period (lower panel) are shown for the same animals. Each data column is averaged (±SEM) over 5 weekdays during the second week of each condition and over six animals. *Significant difference between means in posthoc tests ($p < 0.05$).

at midday. (This schedule was different for each animal.) Inactive periods occurred between 6 p.m. and 6 a.m.

Drug Effects

The total number of inactive periods/day was significantly increased according to drug condition, $F(4,16) = 4.50$, $p = 0.01$ (Fig. 2). Total inactive periods/day increased above (i.e., activity density decreased below) baseline levels during diuretic alone and increased further during diuretic + verapamil combined. On average during the combination dosing period, there were an additional two 30-min periods with zero activity/day over baseline levels. The total number of inactive periods/day fell toward baseline levels in the subsequent conditions of verapamil alone and baseline recovery.

Figure 2 also displays data for number of consecutive inactive periods/day, which showed a similar, near-significant trend across drug conditions, $F(4,16) = 2.93$, $p = 0.06$. The average number of consecutive 30-min periods with zero activity increased slightly from 1.5 per day during baseline to 2 per day during the combination dosing condition. Activity density/day was not changed as a function of drug conditions ($p > 0.05$).

Drug Effect as a Function of Time of Day

As described above, the drug combination dosing period altered activity levels by increasing the number of periods with zero activity. A further analysis of within-day activity directly compared the combination drug dosing period with the baseline period across three fixed segments of the day. These segments were selected to be periods 4 h long, each separated by 4 h, representing distinctly different parts of the day starting 1 h after lights on: morning (0700–1100 h), afternoon (1500–1900 h), and evening (2000–2400 h). The morning time included arrival of laboratory staff; the evening time was during a lights-out period that includes sleeping time.

A main effect of drug conditions was evident, $F(1,4) = 31.29$, $p = 0.005$, such that for these time periods averaged activity during drug combination was 0.169 (±0.062) as compared to baseline activity of 0.200 (±0.067), a decrease of approximately 16%. There also was a significant interaction

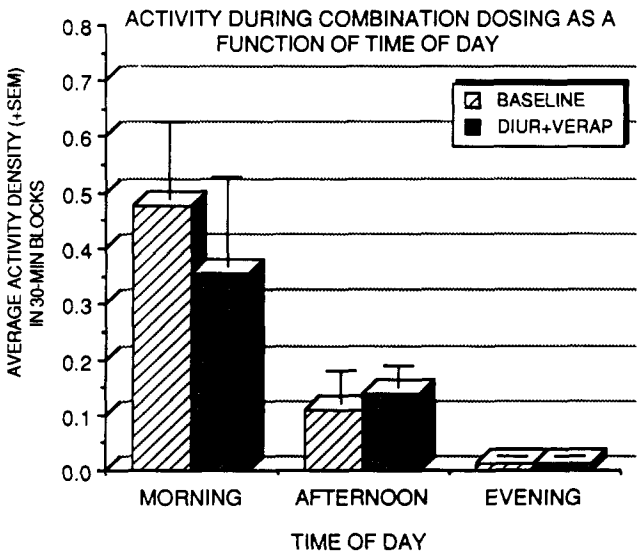

FIG. 3. Activity density as a function of time of day, showing activity during baseline (light bars) and diuretic + verapamil combination dosing (dark bars). For each time of day, activity in 30-min bins was averaged over a 4-h period: morning, 0700–1100 h; afternoon, 1500–1900 h; evening, 2000–2400 h. Each data column is averaged (±SEM) over 5 weekdays during the second week of each condition and over six animals. The condition × time of day interaction was significant at $p < 0.05$. Individual means comparisons were not significant.

of drug condition × time of day, $F(2,8) = 8.46$, $p = 0.025$. The largest effect of combination dosing on activity was in the 0700–1100 h (morning) period, during which activity levels were reduced by approximately 25% as an average for all animals (Fig. 3).

The sleep fragmentation index was not statistically significant for any main effects or interactions. Finally, there were no significant main effects of hypertension group for any of the activity analyses.

DISCUSSION

The results of this study showed that the combination of verapamil and diuretic significantly decreased spontaneous activity. During the second week of chronic oral dosing, the combination of verapamil and diuretic increased both the number and duration of immobile periods and selectively decreased activity levels during the morning hours in comparison to afternoon and evening periods of the day. A similar but not significant trend was observed for the diuretic alone. During verapamil alone and during posttreatment placebo, activity levels generally returned toward those observed at baseline. There were no significant differences between baseline and postdrug placebo conditions.

The amount of activity decrease was notable, resulting in about an hour more of inactivity per day and a 20% decrease during the morning. The morning decrease occurred during the most active period of the day for the animals and may therefore represent either some overall decrease in activity more clearly seen during the most active period or some subtle change in circadian rhythm. Note that the decreases in activity occurred in the morning hours despite the staggered dosing times for animals across the day. When evaluated across the entire day, however, overall activity was not changed across treatment conditions; it thus seems unlikely the decreases could be considered a general effect on activity. A change in the circadian pattern of activity seems more likely, with some persistence of inactivity from the normal rest period into the morning. The times of the primary rest periods remained linked to the light–dark cycle; however, the large increase in activity in the morning seemed blunted with persistence of inactivity from the sleep period into the morning time.

We have reported earlier that diuretic dosing alone did not produce hypokalemia, nor were behavioral performances impaired in these animals (25). Activity levels assessed here also were not changed during diuretic dosing alone. We have, however, previously found the calcium antagonists to impair behavioral performances (23), leading to the present assessment of the effects of calcium antagonists on general activity. Verapamil, a papaverine-derivative calcium antagonist, has been associated with fewer complaints of headache and flushing than the prototypical calcium antagonist nifedipine, which is a stronger vasodilator (13). In a comparison of 16 calcium antagonists, Grebb (8) found that the calcium antagonist nifedipine but not verapamil blocked amphetamine-induced locomotor stimulation. In this study, verapamil did not affect spontaneous activity when administered alone.

During the 11-week activity measurement period of the present study, these baboons also experienced daily matching-to-sample behavioral testing sessions and were measured noninvasively for changes in blood pressure (25). Consistent with the decreases in spontaneous activity found during the diuretic + verapamil dosing period, behavioral performances during experimental sessions also were impaired to a small degree during combination dosing. Importantly, the period of maximal decreases in activity reported here coincided with the period of maximal decrease in blood pressure and maximal behavioral performance impairments (25). This result provides evidence that diuretic + verapamil combination dosing exerts its effects across a variety of behavioral dimensions including sensory discrimination and spontaneous activity, and that these changes accompany the expected time course of antihypertensive effects of these agents. That such changes occurred within the first 2 weeks of combination dosing lends support to the many clinical reports of confusion, memory loss, and sedation within the first weeks of antihypertensive pharmacotherapy, particularly with calcium antagonists (16). Such early side effects often prevent patients from continuing with prescribed medications.

No differences in activity patterns or drug effects were found as a function of hypertension group; this result parallels our previous findings that the groups also did not differ with respect to the magnitude of decrease in blood pressure across drug conditions (25). Activity level and other behavioral comparisons may be more profitably made between animal models of hypertension that are differentially sensitive to calcium antagonists vs. diuretics.

Combined antihypertensive medications may produce side effects not observed during monotherapy with either drug. These effects, while behaviorally significant, are not as marked as large changes in activity typically induced by the sedative and opiate classes of drugs [e.g., (10)], suggesting the need for sensitive behavioral assays in the investigation of antihypertensive agents. Many other compounds such as beta-blockers, angiotensin-converting enzyme inhibitors, and vasodilators are combined with diuretics for treatment of hypertension, but their combined behavioral side effects have not been studied in the laboratory. A nonhuman primate model in the study of antihypertensive drugs thus may be profitably employed in examining the source of therapeutic dropouts due to reported side effects. In this regard, the present data show that activity monitoring itself provides a useful behavioral assay for drug effects that in this study corresponds to clinically reported side effects of fatigue or sedation.

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