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Sustained Synergism by Chronic Caffeine of the Motor Control Deficit Produced by Midazolam

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LAU, C. E. AND J. L. FALK. *Sustained synergism by chronic caffeine of the motor control deficit produced by midazolam.* PHARMACOL BIOCHEM BEHAV **40**(4) 723–731, 1991.—To evaluate the effects of chronic caffeine on the impairment of discriminative fine motor control produced by midazolam, rats were trained to hold a force transducer steady to deliver food pellets. Chronic, daily doses of midazolam (3 mg/kg SC) led to a stable level of motor impairment. Chronic caffeine (20 mg/kg IP) alone usually produced a more moderate deficit or, for one animal, no deficit. Combined, chronic administration of these doses yielded a sustained synergism in motor performance impairment, which contrasted with the antagonism usually found between the benzodiazepines and methylxanthines when performance is evaluated by psychomotor tests not requiring fine motor control. The observed synergism was not explicable in terms of measured disposition of the drugs. The synergistic production of fine motor dyskinesia by the concurrent administration of caffeine and midazolam may be relevant to the triggering of anxiety attacks by caffeine observed in panic disorder patients.

Midazolam and caffeine complications Psychomotor performance Methylxanthine-benzodiazepine interaction
Panic disorder Midazolam pharmacokinetics Caffeine pharmacokinetics

MIDAZOLAM is a benzodiazepine possessing a rapid onset and short duration of its pharmacodynamic actions (33). Acute doses of midazolam (0.75–3 mg/kg SC) produced impaired performance on a discriminative fine motor control task in rats (22, 44, 48). In this task, rats were trained to hold a force transducer operated with a paw so that it remained within the upper and lower limits of a force band for a continuous 1.5-s period to deliver each of 50 food pellets. The performance impairment produced by midazolam was antagonized in a dose-related fashion by Ro 15-1788, a competitive benzodiazepine antagonist (22).

Benzodiazepine agents are used for a variety of therapeutic purposes, some of which (sedative, anticonvulsant and muscle-relaxant) aim specifically at producing changes in motor behavior. At therapeutic dose levels, these agents also can result in acute impairment of human psychomotor performances (52). This impairment is synergized by the concurrent administration of ethanol (26,45). On the other hand, studies of the effect of caffeine on benzodiazepine pharmacodynamics usually find that caffeine antagonizes the psychomotor impairment and anxiolytic actions of benzodiazepines (45). It has been suggested that some of the actions of the benzodiazepines may be due to their blockade of cellular uptake of adenosine in the brain, thereby increasing the depressant effect of adenosine, and that the blocking effect of methylxanthines on adenosine receptors could account for methylxanthine antagonism of benzodiazepine action (32). An in vivo receptor-binding study did not support the possibility that caffeine-benzodiazepine antagonism is due to an interaction at the benzodiazepine binding site (34). In previous studies, we found that, although the acute administration of caffeine acted to antagonize the anxiolytic effect of clonazepam (43), it markedly synergized the impairment produced by midazolam on discriminative fine motor control (12). Benzodiazepine-caffeine synergism was an unexpected finding. One aim of the present study was to extend the findings on midazolam-caffeine synergism beyond acute dose combinations to an analysis of chronic, concurrent administration.

It is possible that the observation of a midazolam-caffeine synergism might be explained by some change in pharmacokinetics. The presence of one drug might alter the kinetics of the other agent in such a way that its effectiveness for disrupting motor performance is increased. Thus a second aim was to determine whether acute or chronic administration of either midazolam or a midazolam-caffeine combination affected drug and metabolite disposition at the postinjection time when motor performance was being evaluated.

METHOD

Animals

Seventeen male albino rats of the Holtzman strain (Madison, WI) with a mean initial body weight of 385 g (range: 377–389 g; approximately 80 days old) were used. They were housed individually in a temperature-regulated room with a daily cycle of illumination from 7:00 a.m. to 7:00 p.m. They were reduced to 80% of their ad lib body weights over a 2-week period by limiting daily food rations, and held at these weights for the duration of the experiments. Water was continuously available in the living cages. For the five animals trained on the discriminative

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motor control procedure, food supplements necessary for maintaining these weights were given daily in the living cages immediately after experimental sessions during which food pellets were presented contingent upon performance on a motor task. All aspects of the experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

Drugs

The administered drugs were: 1) midazolam maleate, dissolved in nanopure water administered SC and 2) caffeine (Sigma Chemical Co., St. Louis, MO) dissolved in a sodium benzoate (37.5 mg/ml) solution administered IP. Drug doses of midazolam are expressed in terms of the salt, while caffeine doses are given as the base.

Apparatus

The experimental space was a Plexiglas chamber (25 × 30 × 30 cm) with stainless steel front and rear panels and a floor consisting of parallel-mounted, spaced, stainless steel rods. Discriminative motor control was measured using a force-sensitive transducer connected to a stainless steel operandum mounted on the front panel 2.5 cm from the floor. The operandum was surrounded by a thick Plexiglas shield fashioned with a 1.0 cm wide × 4.0 cm high slot so that access to it was limited to a single paw. A previous publication gives a more complete description of the apparatus (12). The voltage output from the force transducer was conveyed to a customized signal control box and sorted into one of three signal regions: above, below or within a window defined by preset lower and upper voltage limits. These limits corresponded to applied forces of 0.147 N (15 g force) and 0.265 N (27 g force), respectively, incident at the paw-placement region of the operandum. A buffer was set so that a minimum force of 0.015 N (1.5 g force) was required for signal recognition. An Apple IIe microcomputer was programmed in assembly language to sample signal input once every 10 ms. When the force applied by the animal was within the 0.147 to 0.265 N band, an audio feedback signal (Sonalert SC648H, P. R. Mallory, Indianapolis, IN) was turned on.

Discriminative Motor Control Measures

The training sequence for producing the final discriminative motor control performance has been described previously (9). A continuously applied in-band force lasting 1.5 s was required for the delivery of a 45-mg food pellet (Bio Serv, Inc., Frenchtown, NJ). If the applied force went above or below the band before 1.5 s had elapsed, then this timer was reset. Thus the behavior reinforced by food pellet delivery was holding the force transducer steadily operated within the force band for a continuous, set period of time. Ordinarily, a session was terminated when the 50th pellet had been delivered, but a session also was terminated if 30 min had elapsed without operation of the transducer.

The raw measures of motor behavior taken for each session were: the *session time* (the time taken to earn 50 pellets), the *total response time* (amount of the session time that the transducer was held operated above the minimum recognition threshold of 0.015 N), the *in-band time* (amount of the session time that the transducer was held operated within the force band, i.e., between 0.147 and 0.265 N), and the *entrances* (the total number of times during a session that the applied force entered the band from either the lower or upper set limits). Except in the case of the entrances measure, these raw measures in isolation are not useful characterizations of motor performance. For example, the in-band time measure is best interpreted in relation to how it compares with the minimum total in-band time that would satisfy the contingencies set for a particular experiment (e.g., in the present case, this value is 1.5 s/pellet for a total of 50 pellets, which yields a minimum possible in-band time of 75 s). Similarly, raw session in-band time is difficult to interpret unless viewed in relation to total response time.

Four measures of motor behavior were calculated from each session:

$$\text{In-band efficiency} = \frac{\text{minimum possible in-band time}}{\text{in-band time}}$$

$$\text{Tonic accuracy} = \frac{\text{in-band time}}{\text{total response time}}$$

$$\text{Work rate} = \frac{\text{total response time}}{\text{session time}}$$

Entrances = total number of entrances into the force band.

The in-band efficiency measure has a fixed numerator (50 pellets × 1.5 s), making the minimum possible time in-band to deliver all pellets 75 s. A perfectly efficient performance would yield an efficiency measure of 1.00. The measure of tonic accuracy approaches 1.00 as the total time spent responding (i.e., more than 0.015 N applied to the transducer) approaches the time spent in-band. It measures an aspect of discriminative motor control that is somewhat different than that measured by in-band efficiency. Although a high proportion of session operandum-holding might be within the appropriate force band, if the holding times are frequently of too short a duration to produce pellet delivery, then tonic accuracy could be high although in-band efficiency is low. Work rate is simply the proportion of the session time that the animal spends operating the transducer. Because work rate can approach a value of 1.00 or zero, the previous measures can approximate 1.00 or zero in complete independence of work rate. The entrances measure is simply the number of times the applied force enters the appropriate band, with a high count indicating difficulty maintaining steady in-band holding. It is a different measure than in-band efficiency, in which relative inefficiency could indicate that the in-band hold times often fall just short of the appropriate hold time; such a performance would not yield a high entrances measure.

Reagents, Serum Sampling and HPLC

All analyses were performed using a Waters Associates (Milford, MA) 510 pump, equipped with a Reodyne (Cotati, CA) Model 7010 sample injection valve with a 20-μl loop, and a Beckman (San Ramon, CA) variable wavelength detector 163. Absorbances at 230 nm for midazolam and 270 nm for caffeine and its metabolites were monitored on a Perkin-Elmer (Norwalk, CT) integrator LCI-100. The separations for both drugs were performed on an Altex (San Ramon, CA) Ultrasphere C18 column (5-μm particle size, 150 × 2.0 mm i.d). A Reodyne 2-μm precolumn filter also was used. Diethyl ether and ethanol were HPLC grade and obtained from Aldrich Chemical (Milwaukee, WI). HPLC-grade chloroform, acetonitrile and methanol were purchased from Fisher Scientific (Springfield, NJ) and tetrabutylammonium phosphate from Eastman Kodak Co. (Rochester, NY). All other chemicals were reagent grade. The 1 M borate-sodium carbonate-potassium chloride buffer (pH 9.0) was prepared as described by de Silva and Puglisi (11). Midazolam

maleate was obtained from Hoffmann-La Roche (Nutley, NJ) and caffeine, theobromine, paraxanthine, theophylline and β-hydroxyethyltheophylline from Sigma Chemical Co. (St. Louis, MO). An aqueous stock solution of midazolam at 1.0 mg/ml (free base) was prepared, and working standards of midazolam (0.05, 0.1, 0.25, 0.5, 1.0 and 2.0 μg/ml) were made by appropriate dilutions of the stock solution with drug-free rat serum. Separate aqueous stock solutions of caffeine, theobromine, paraxanthine, theophylline and β-hydroxyethyltheophylline also were prepared at a concentration of 1.0 mg/ml. Working standards of caffeine and the three metabolites (0.05, 0.1, 0.25, 0.5, 0.75 and 1.0 mg/ml) were prepared by appropriate dilutions of the stock solutions with drug-free rat serum. The internal standard, β-hydroxyethyltheophylline, was used at a concentration of 2 μg/ml. The mobile phase for midazolam was methanol-acetonitrile-33 mM sodium acetate buffer containing 0.83 mM tetrabutylammonium phosphate adjusted to pH 2.9 with 40% phosphoric acid (12:25:60, v/v/v). The flow rate was set at 0.3 ml/min and normally operated at a pressure of 138 bar (2000 p.s.i.). The mobile phase for caffeine and its metabolites was methanol-1% acetic acid-28 mM sodium acetate buffer containing 1.4 mM tetrabutylammonium phosphate adjusted to pH 2.15 with 40% phosphoric acid (12:52:36, v/v/v). The flow rate was set at 0.3 ml/min and operated at a pressure of 103 bar (1500 p.s.i.).

Serum samples were prepared as previously described (21). Fifty μl of midazolam working standard was mixed with 1 M borate buffer pH 9.0 (100 μl) in a 15-ml conical centrifuge tube. Then, diethyl ether (2.5 ml) was added, and the result mixed and centrifuged. For preparing caffeine and its metabolites, 25 μl of internal standard, β-hydroxyethyltheophylline (2 μg/ml), was added to 50 μl of working standard and mixed with borate buffer (100 μl) and 1 ml of chloroform:ethanol (82.5:17.5). Samples for serum drug analysis were prepared identically except drug working standards were not added.

Procedure

Five animals were given discriminative motor control sessions at the same time every day. After about 4 months, intersession performance had stabilized and drug dosing began. After receiving acute doses of midazolam, caffeine and midazolam-caffeine combinations, the effects of which on motor control performance were described in a previous report (12), chronic dose levels were selected. Three animals received chronic dosing with midazolam first; two received chronic caffeine first. The two dosing sequences were as follows: Animals J1, K14 and G10 were injected daily, 30 min presession, with 3 mg/kg midazolam SC for 50 to 54 days. This was followed by daily administration of a combined midazolam-caffeine dosing regimen (M+C) for 15 to 28 days. The M+C combination consisted of a continuation of the above midazolam dosing procedure with the addition of a 20-min presession, daily 20-mg/kg caffeine IP dose. Animals were then returned to the presession administration of midazolam alone for 22 to 39 days. This was followed by 10 days of presession midazolam-vehicle injection SC, and 45 to 79 additional session days without injections to allow for complete recovery from chronic drug administration. Then, animals were injected daily 20 min presession with caffeine 20 mg/kg IP for 21 to 23 days. This was followed by 10 days of presession caffeine-vehicle injection IP, and 10 additional session days without injections to allow a complete recovery of baseline conditions.

Animals G8 and G16 were injected daily, 20 min presession, with 20 mg/kg caffeine IP for 26 and 21 days, respectively. This was followed by 10 days of presession caffeine-vehicle injection IP and 65 and 90 additional session days without injections to allow for complete recovery from chronic caffeine administration. Then G8 and G16 were injected daily, 30 min presession, with 3 mg/kg midazolam SC for 26 and 21 days, respectively. This was followed by daily presession injection of the M+C combination for 22 days (G8) and 24 days (G16) and a return to the presession administration of midazolam alone for 9 and 14 days, respectively. This was followed by 10 days of presession midazolam-vehicle injection SC, and 10 additional session days without injections to allow a complete recovery of baseline conditions. In all cases, animals were not advanced from one experimental condition to the next one until an individual's interday data were deemed stable. Hence, as indicated by the above description, the number of days animals remained in each condition was varied appropriately.

In order to determine whether acute or chronic administration of the midazolam dose, the caffeine dose, or the midazolam-caffeine combination affected drug or caffeine metabolite disposition at the postinjection time when motor performance was being evaluated, three groups of animals (N=4 each), maintained at 80% body weight, were used. A Midazolam Group received an acute injection of 1.5 mg/kg midazolam SC and, 7 days later, a 3-mg/kg dose. A tail-tip blood sample (100 μl) was obtained for serum drug analysis 30 min after the second injection. Animals then received daily 3-mg/kg midazolam injections, and blood samples were taken 30 min after the 47th, 57th and 67th injections. A Caffeine Group received acute injections of 10, 20 and 40 mg/kg caffeine IP 5 to 7 days apart. A tail-tip blood sample was taken 20 min after the second injection. Seven days later, daily 20-mg/kg caffeine injections began, and blood samples were taken 20 min after the 1st, 11th and 21st administrations of this dose series. An M+C Group received acute injections of 20 mg/kg caffeine and 3 mg/kg midazolam 7 days apart, and corresponding serum samples were taken at 20 and 30 min postinjection, respectively. After 7 days, animals received daily midazolam injections for 46 days and then the combined, daily administration of midazolam and caffeine was started. As was the case for the M+C phase of the discriminative motor control experiment, the midazolam injection preceded the caffeine injection by 10 min. Blood samples for the measurement of serum concentrations of midazolam and caffeine were taken 20 min after the caffeine injection on the 47th, 57th and 67th days of chronic midazolam injection, i.e., on the 1st, 11th and 21st days of the chronic M+C procedure.

RESULTS

Figure 1 shows overall results for the five animals trained on the discriminative motor control task. Each quadrant of Fig. 1 (2nd bar from the left, labeled M) shows group mean values for the last 5 days of chronic midazolam (3 mg/kg SC) administration for the initial series of sessions in which animals were exposed chronically to midazolam. The individual-animal means are indicated by unique symbols. Relative to baseline values (cf. left bar, labeled B; 5-day mean of values preceding start of chronic midazolam injection), four of the five animals had appreciable decreases in in-band efficiency and increases in entrances. Performance deficit also was reflected by a decrease in tonic accuracy. Group work rate was little affected. The mean values for the entire period of daily caffeine administration (cf. 3rd bar from left, labeled C), which continued for a period of between 21 and 26 days, showed the same pattern of results, except that the mean performance deficit was less severe than was the case under chronic midazolam administration. Mean values for the entire period of daily exposure to the concurrent

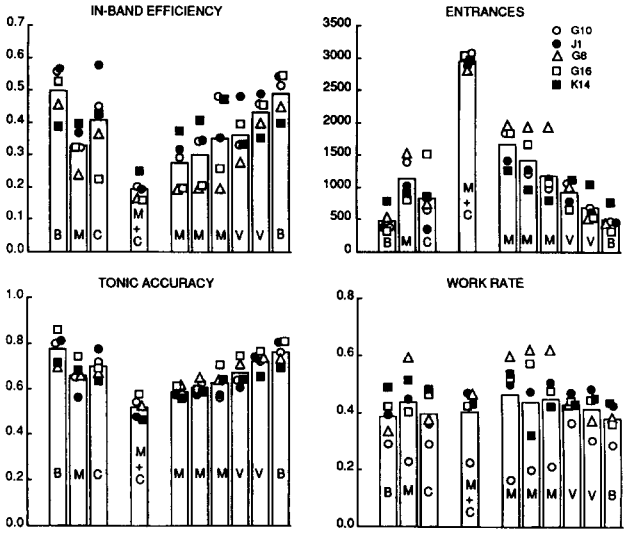

FIG. 1. Mean values for 5 animals (symbols) for 4 indices of discriminative motor control performance. From left to right in each quandrant, B: baseline; M: chronic 3 mg/kg SC midazolam; C: chronic 20 mg/kg IP caffeine; M+C: chronic 3 mg/kg midazolam + 20 mg/kg caffeine; M: return to chronic 3 mg/kg midazolam (consecutive 5-day means); V: substitution of vehicle injection for midazolam (consecutive 5-day means); B: recovery of baseline (10-day mean).

combination of midazolam and caffeine (cf. bar labeled M+C; comprising a period of between 15 and 28 days) produced a synergistic increase in motor performance deficit with no change in work rate. (G10 did not finish the session on three of these occasions. These sessions are not part of the calculated mean and will be considered below.) When the daily midazolam injections were continued, but with discontinuation of the daily caffeine injections (cf. consecutive 5-day mean value bars labeled M), motor performance progressively returned to the previous midazolam-alone level. (G8 remained in this treatment phase for only 9 days, at which time its performance was deemed to have returned to the previous midazolam-alone level, and the next phase of the experiment was begun for this animal. Consequently, for G8, the value at the 2nd of these M bars is based on only four sessions, rather than on five, and the value in the next M bar is an assumed iteration of the previous value.) Upon substitution of daily vehicle injections, in place of midazolam injections (cf. consecutive 5-day mean value bars labeled V), performance again progressively improved and finally recovered to the previous baseline level with additional daily sessions (cf. 10-day mean bar labeled B on right). As indicated in the procedure, three animals received chronic dosing with midazolam first; two received chronic caffeine first. The two dosing sequences had no differential effect on the results produced by either drug alone or by M+C.

Figure 2 presents a more detailed picture of selected aspects of the results for two of the motor performance measures. Included are consecutive, daily sessions for all the caffeine (open circles) and M+C (filled circles) sessions, as well as the first five vehicle sessions after drug discontinuation for both the caffeine injection series (open squares) and the final midazolam injection series (filled squares). These consecutive vehicle sessions are presented to enable the detection of possible withdrawal effects upon discontinuation of the chronic midazolam and chronic caffeine administration series.

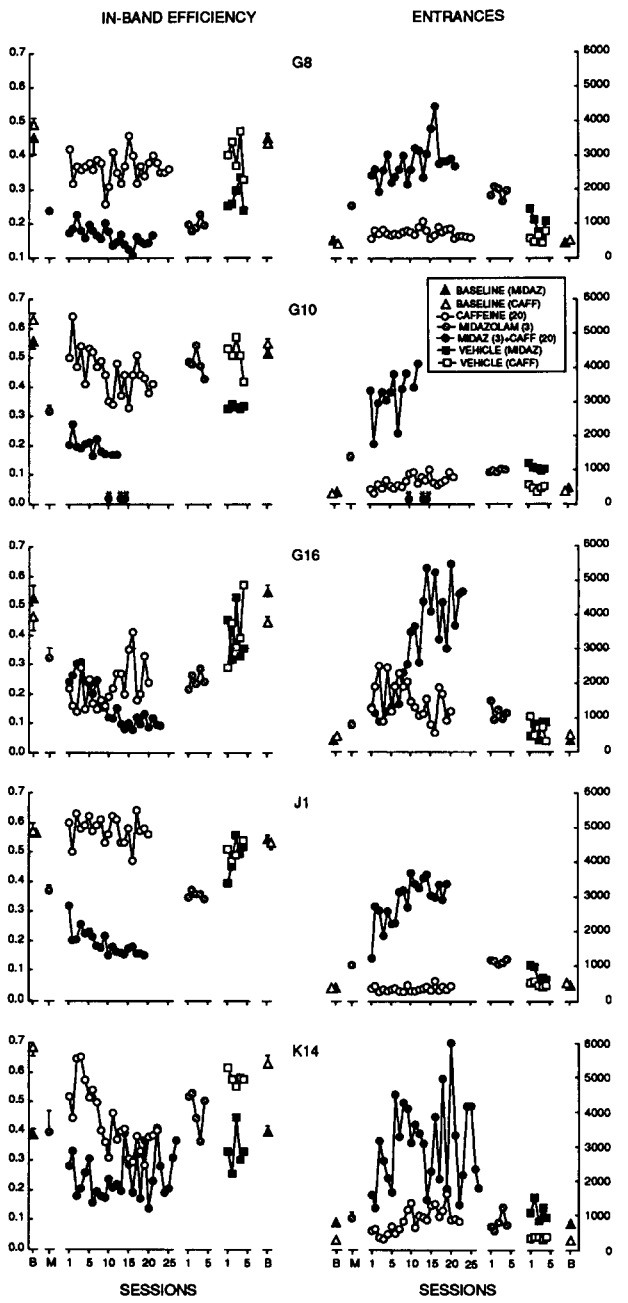

FIG. 2. Values for consecutive sessions for each of 5 animals for 2 indices of discriminative motor control performance before, during, and after chronic drug and drug combination conditions. Filled and open triangles (leftmost points of each half of figure): 5-day baseline means (S.E.) before start of chronic 3 mg/kg SC midazolam or 20 mg/kg IP caffeine, respectively; open circles: all chronic caffeine sessions; open squares: first five vehicle sessions after caffeine discontinuation; partially filled circle above M symbol: mean (S.E.) of last 5 sessions of chronic midazolam before M+C started; filled circles: all chronic M+C sessions (asterisks near abscissa for G10 represent sessions animal did not finish.); partially filled circles: last 5 sessions of chronic midazolam after chronic caffeine withdrawn from M+C condition; filled squares: first five vehicle sessions after midazolam discontinuation; filled and open triangles (rightmost points of each half of figure): recovery of baseline condition (10-day means, S.E.s lie within plotted points) after chronic midazolam or chronic caffeine drug sessions and their postdrug vehicle sessions had been completed.

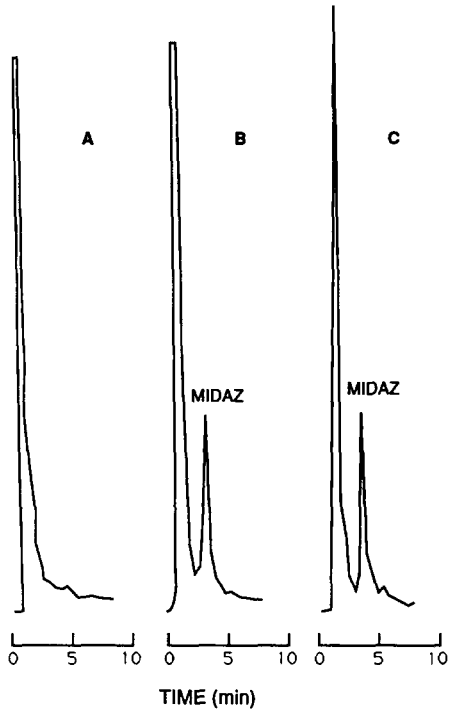

FIG. 3. Chromatograms of (A) rat serum blank, (B) spiked serum sample containing 0.25 μg/ml midazolam standard, and (C) serum sample obtained after 3 mg/kg midazolam SC injection.

The leftmost points of each half of Fig. 2 (above label B) show the mean 5-day values for baselines preceding the start of the chronic caffeine (open triangles) and the chronic midazolam (filled triangles) injection series. As indicated in the preceding section, these baseline determinations were separated by about six months, during which animals were exposed to other phases of the experiment. Although this lengthy period of daily practice led to no major shifts in baseline performance for most of the animals, K14, which was one of the animals that received chronic caffeine-alone administration as a late phase of the experiment, showed a noticeable improvement in both in-band efficiency and entrances.

In comparison, then, to the leftmost baseline values (open triangles), chronic caffeine administration, for which all sessions are shown, produced performance deficits in all animals except J1. Of the four animals affected by daily caffeine injection, two (G8 and G10) were more severely affected by the daily midazolam dosing than by caffeine dosing (cf. mean of last 5 midazolam days before start of M+C combination, plotted point above label M), one (G16) was affected about equally, and one (K14) was unaffected. J1 showed a performance deficit under daily midazolam injection comparable to the other three affected animals, but was unaffected by daily caffeine administration.

The chronic M+C combination, for which all sessions are shown, produced a synergistic action on the behavior of all animals and resulted in the most severe deficits on both measures of motor performance shown in Fig. 2 (filled circles). When G10 was so severely affected by the M+C combination that it began not to finish sessions (cf. filled circles plotted near abscissa with asterisks), it was advanced to the next phase of the experiment. It is of interest that J1, which was unaffected by chronic caffeine administration, nevertheless exhibited the synergistic action of the M+C combination displayed by the other animals.

Upon discontinuation of the daily caffeine dose component from the M+C combination, motor performance improved to approximately the previous midazolam-alone level [compare the last five midazolam sessions (partially filled circles) with the mean points plotted above M].

There was no indication of a disruptive caffeine withdrawal effect on motor performance when daily caffeine vehicle injection replaced caffeine injection (compare open circles with open squares, Fig. 2). Likewise, when daily vehicle injection replaced midazolam injection, there was little indication of a short-lived, disruptive effect that might be expected from withdrawal of this agent (cf. transition from partially filled circles to filled squares). The recovery of noninjection behavior baselines (10-day means) directly after both the postmidazolam vehicle and postcaffeine vehicle injection series is shown at the rightmost plotted points above the B symbol.

Figures 3 and 4 show the chromatograms of serum blanks (A), spiked serum samples of midazolam standard, or the standard for caffeine and its metabolites containing the internal standard (B), and rat serum tail-tip samples obtained after 3 mg/kg midazolam SC or 20 mg/kg caffeine IP (C). Midazolam samples from the M+C Group did not interfere with the determination of caffeine and its metabolites; the converse was also the case. Mean recovery of midazolam was 94 (3.3)%. The coefficients of variation for midazolam were 2.5% and 5.2% for within-day and between-day precisions, respectively. Midazolam peak heights were linear within the range examined (0.05–2.0 μg/ml). Mean recoveries were: caffeine 82.9 (6.6)%, theobromine 91.5 (2.5)%, paraxanthin 53.2 (1.1)% and theophylline 63.6 (2.2)%. The coefficients of variation for these compounds ranged from 1.0–7.7% for within-day and 1.0–5.7% for between-day precision. The ratio of caffeine and its metabolites to the internal standard was linear within the range examined (0.05–5.0 μg/ml).

Figure 5 shows the mean serum drug concentration values at the postinjection time corresponding to the start of a discriminative motor control session (20 min postinjection for caffeine and its metabolites and 30 min for midazolam). Serum concentration values are shown for caffeine and three metabolites after the acute administration of caffeine (20 mg/kg IP) and for midazolam after acute administration of midazolam (3 mg/kg SC) (Fig. 5, leftmost pair of bars for each agent). Serum values were measured during chronic administration after the indicated numbers of days of caffeine dosing (Caffeine Group) and midazolam dosing (Midazolam Group and M+C Group), as described in the Method section. One-way ANOVA of serum values for the Caffeine Group indicated that chronic caffeine administration led to an increased accumulation of caffeine ($p<0.0001$), theophylline ($p<0.01$), theobromine ($p<0.01$) and paraxanthine ($p<0.02$). Chronic caffeine administration in the M+C Group, which had received chronic midazolam administration over the preceding 46 days, also led to an increased accumulation of caffeine ($p<0.02$), theophylline ($p<0.01$), theobromine ($p<0.0001$) and paraxanthine ($p<0.001$). A 2-way ANOVA comparison of these two groups for the 11- and 21-day values showed that the accumulations of caffeine ($p<0.0001$) and theophylline ($p<0.01$) were significantly less for the M+C Group than for the Caffeine Group. Theobromine and paraxanthine accumulation levels were not different between the groups. Chronic administration of midazolam did not lead to significant changes in serum midazolam levels for either the Midazolam Group or the M+C Group, nor were the levels significantly different between the groups (Fig. 5, bottom).

DISCUSSION

The impairment in discriminative motor control produced by

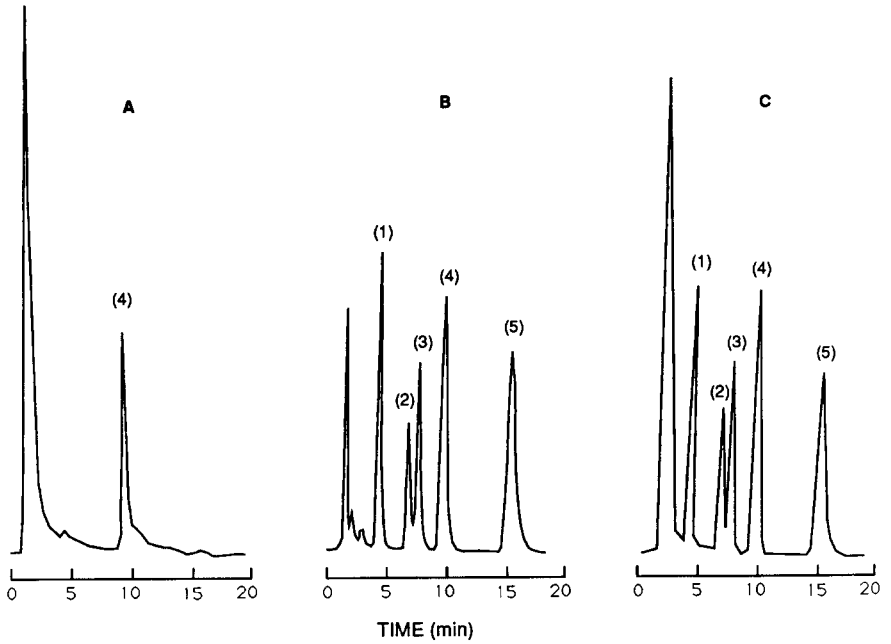

FIG. 4. Chromatograms of (A) rat serum blank with internal standard, (B) spiked serum sample containing caffeine (1.0 μg/ml) and its metabolite standards (0.5 μg/ml) with peaks (1) theobromine, (2) paraxanthine, (3) theophylline, (4) β-hydroxyethyltheophylline internal standard, (5) caffeine, and (C) serum sample obtained after 20 mg/kg caffeine IP with same peak identifications.

the chronic administration of 3 mg/kg SC midazolam was similar to the impairment reported in our previous studies for this dose (22, 44, 48). Caffeine, at the dose administered in the present experiment, has produced increases in spontaneous motor activity (14, 31, 39, 46) and in operant response rates (28, 30, 38) in rats. With regard to discriminative motor control, caffeine impaired hand steadiness performance in humans (15,24). Several studies, which required subjects to hold a metal stylus in a hole without touching the sides, found decreased steadiness after one cup of coffee was ingested, or after subjects had received caffeine doses equivalent to 3 or 4 cups (51).

In a previous report, animals used in the present study received acute doses of caffeine (10 to 40 mg/kg), but the motor performance of only one (K14) was affected appreciably (12). It was affected by the acute 10-mg/kg caffeine dose and failed to finish sessions when it received either the 20- or 40-mg/kg dose. It did finish all sessions in the present study that were preceded by the 20-mg/kg caffeine dose, indicating that tolerance had developed to the disruptive effect of this dose of caffeine. On the other hand, with the exception of J1, the remaining animals now were affected by this dose of caffeine and showed no development of tolerance with daily dosing. The sensitivity of most of the animals to the 20-mg/kg caffeine dose under daily dosing, in contrast to the relative lack of effect of the acute dose, and indeed, the increasing sensitivity of G10 and K14 with repeated caffeine doses (Fig. 2), indicates that perhaps both tolerance and sensitization phenomena can occur concurrently with respect to discriminative motor control at this dose level of caffeine. Marked tolerance to the repeated administration of caffeine is known to develop in rats with respect to operant performances (5,49), caffeine discriminative-stimulus effects (17) and locomotor activity (17,40), and in humans to the cardiovascular effects of caffeine (37). In the matter of sensitization to caffeine, intermittent-dose regimens, in comparison to daily administration, are more efficacious in producing sensitization to motor activity enhancement by psychomotor stimulants such as cocaine and the amphetamines (35,42). Most studies, including the present one, investigating chronic caffeine effects have exposed subjects on a daily basis. However, wheel running in rats was reported to increase to a higher level with alternate-day availability of caffeine for drinking than it did under a condition of daily availability (29). In the M+C series, caffeine had a conspicuous synergistic effect on the motor impairment produced by midazolam. This result contrasts with a great part of the literature on the interaction of the benzodiazepines with the methylxanthines. Although some studies have reported additive, anticonflict action when a benzodiazepine and caffeine were coadministered (1, 7, 47), others find either no effect or an antagonism (8, 34, 43). In mice, IP caffeine doses increased locomotor activity, and this increase was antagonized by alprazolam (19). Furthermore, the impaired performances produced by benzodiazepine administration on a variety of psychomotor tests were antagonized by the coadministration of caffeine (13, 15, 24, 36) or theophylline (16,27). It is of interest, however, that hand steadiness performance impaired by caffeine was not antagonized by diazepam (15,24), but neither was it synergized. With respect to motor function, only a brief report on locomotor activity in mice indicated synergistic action: The combination of a depressant dose of diazepam with an inactive dose of caffeine produced an increase in activity (20). In a recent study, acute cocaine, as well as acute caffeine doses, synergized the motor control impairment produced by midazolam (12). We suggest that the fine motor control requirement in these experiments may be an important feature for revealing the observed synergism. Many of the motor performance tasks used in testing humans evaluate the speed of performance of simple, repetitive acts. These may be more useful for detecting the sedative component of the benzodiazepines than for evaluating fine motor control capacity; sedative action may be more readily antagonized by psychomotor stimulants than is fine motor dyskinesia (2). Tolerance to the synergism produced by

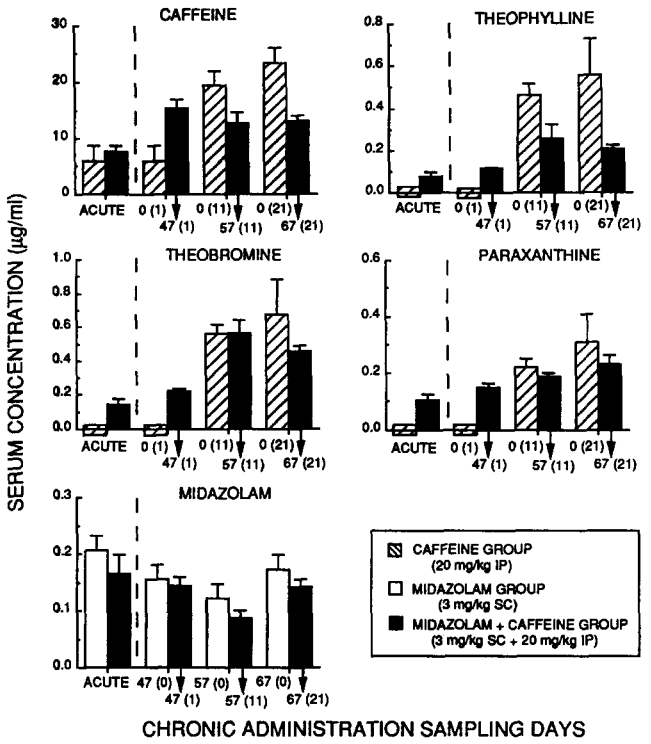

FIG. 5. Mean (S.E.) serum values for caffeine, its metabolites, and for midazolam at the postinjection time corresponding to the start of a discriminative motor control session (20 min postinjection for caffeine and its metabolites and 30 min for midazolam). *Acute*: serum values of: 1) caffeine and 3 metabolites measured after acute administration of caffeine (20 mg/kg IP), 2) midazolam (bottom) after acute administration of midazolam (3 mg/kg SC); *Chronic Administration Sampling Days*: Serum values measured after indicated number of daily injections of caffeine and/or midazolam, where 1st number of pair is the chronic midazolam day and 2nd number, in parentheses, is the chronic caffeine day, e.g., 57 (11) is the serum sample taken after 57 days of midazolam injection and 11 days of caffeine injection. (M+C dosing was preceded by 46 days of daily midazolam dosing, so that daily caffeine dosing started on the 47th midazolam day for this group.)

M+C did not develop. Indeed, as M+C sessions continued, the performance of animals G16 and J1 became increasingly impaired, particularly with respect to the entrances measure (Fig. 2), a result that suggests sensitization development rather than tolerance. In the human studies of benzodiazepine-caffeine interaction cited, only acute dose combinations were administered, so that possible tolerance or sensitization phenomena cannot be evaluated.

The possibility that benzodiazepine-methylxanthine synergism might be explained in terms of methylxanthine effects on benzodiazepine receptor binding is not supported well by current evidence. Caffeine produced no change in ^{3}H-flunitrazepam binding in mouse brain (34). Other investigations found that caffeine and other xanthines produced only a weak inhibition of benzodiazepine binding in mouse and rat brain (25, 41, 50). Still other studies report a transitory increase in the number of benzodiazepine binding sites in mice fed a diet high in caffeine (3), an increase after a single dose of caffeine (18), and a 31% increase after 12 days of daily 75-mg/kg caffeine doses (53). In a recent study, Kaplan et al. (19) found that caffeine did not change benzodiazepine binding in mouse brain. Whatever may be the case at central benzodiazepine binding sites, a decrease could hardly explain synergism, and an increase probably would serve only to mimic a higher drug dose, an effect that leads to work cessation rather than a sustained, but inefficient, performance.

Likewise, pharmacokinetic considerations currently shed little light on benzodiazepine-caffeine interactions. Caffeine and alprazolam mutually antagonized their motor activity effects in mice, but did not alter each other's uptake into brain (19). In the present study, chronic administration of either midazolam or M+C did not lead to significant changes in the disposition of serum midazolam. The chronic administration of M+C led to lower levels of serum caffeine and theophylline than did chronic caffeine alone, an effect that suggests no explanation for either the observed drug synergism with respect to motor control impairment, or the development, in some animals, of sensitization to chronic M+C administration.

There is an interesting relation between the clinical condition manifesting as anxiety attacks (panic disorder) and caffeine. When panic disorder groups were compared to either clinically depressed or normal groups, panic disorder was associated with more coffee abstainers and much lower caffeine intakes (4,23). Furthermore, panic disorder patients report anxiety reactions to the administration of 10 mg/kg caffeine and to as little as one cup of coffee (4, 6, 23). Only one of these studies provides information on the medication status of these patients (4); most of their panic disorder patients were not receiving benzodiazepines. But it is possible, in light of the present finding of M+C synergism in the disruption of motor control, that at least part of the anxiety reaction produced by caffeine in panic disorder is due to the combination of caffeine intake 1) with a currently administered benzodiazepine, or 2) with an endogenous overproduction of a benzodiazepine receptor ligand. The confirmation of such a relation might account for the low caffeine intake of individuals with panic disorder in terms of a conditioned avoidance of the consequences of benzodiazepine-caffeine synergism.

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