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Caffeine Pretreatment: Enhancement and Attenuation of d-Amphetamine-Induced Activity[1]

BRENT C. WHITE AND GEORGE E. KELLER, III

Division of Science and Mathematics, Centre College, Danville, KY 40422

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WHITE, B. C. AND G. E. KELLER, III. *Caffeine pretreatment: Enhancement and attenuation of d-amphetamine-induced activity*. PHARMACOL BIOCHEM BEHAV 20(3) 383–386, 1984.—Caffeine pretreatment was studied for its effects on d-amphetamine-induced locomotor activity. Caffeine (30 mg/kg) was given either simultaneously with or at one of several intervals (0.5, 1.5, 4.5, 12, and 13.5 hours) before d-amphetamine (1.5 mg/kg). Enhancement of d-amphetamine activity occurred with simultaneous and 30 min caffeine pretreatment. However, when given 12 or 13.5 hours before d-amphetamine, caffeine diminished the activity, measured in stabilimeter cages. Several doses of caffeine (7.5, 15, 30, and 60 mg/kg) were given in multiple treatments ending 12 hours before d-amphetamine (1.5 mg/kg) to determine the effective doses for attenuation of d-amphetamine-induced activity. Only 30 and 60 mg caffeine doses reduced d-amphetamine activity while also interfering with body weight gain.

Amphetamine Caffeine Drug interaction Locomotor activity Stimulants Tolerance Toxicity

CAFFEINE pretreatment has been shown to potentiate the effects of a variety of catecholaminergic drugs, including amphetamine [1, 9, 13], apomorphine [9,14], and the anti-parkinsonian drugs, L-Dopa [16] and Piribedil (ET 495) [6]. As a result, several authors have suggested that caffeine may enhance the therapeutic response to these drugs in the pretreatment of parkinsonism [6] and attentional deficit disorder with hyperactivity [13,14]. In addition to therapeutic interactions, nontherapeutic use of caffeine may alter the effects of these and other drugs [19].

These findings underscore the importance of understanding caffeine-drug interactions. The studies reviewed above establish that caffeine given within an hour before a variety of catecholaminergic agonists will enhance some of their effects. We have expanded on these results by exploring the dose and treatment interval parameters of the locomotor activity response to caffeine's interaction with d-amphetamine.

EXPERIMENT 1

Using a 2×2 factorial design, we examined the effect of caffeine pretreatment given 0.5 or 12 hours before d-amphetamine. The former interval is within the range in which caffeine potentiates the effects of several catecholaminergic drugs [6,18]. The twelve-hour caffeine pretreatment interval has been reported to attenuate the activity effects of d-amphetamine [20].

Method

Thirty-two adult, male, hooded rats were habituated for one week to rectangular stabilimeter cages, and then randomly assigned to one of four groups. Two groups were pretreated with caffeine (30 mg/kg, IP, Volume=1 mg/kg) and the other two with sodium benzoate vehicle (30 mg/kg, IP). One caffeine group and one sodium benzoate group was injected 30 minutes before d-amphetamine sulfate (1.5 mg/kg, IP). The other two groups received caffeine or sodium benzoate 12 hours prior to d-amphetamine. Activity counts were cumulated for 3 hours following d-amphetamine. d-Amphetamine injections occurred 4 hours before the end of the 16-hour light phase of the daily cycle. Stabilimeter cages (20×37×17 cm) pivoted on a central fulcrum, activating counting circuitry. Opaque barriers prevented visual contact but not auditory or olfactory cues. Unilab Chow and water were available throughout the experiment.

Results

Figure 1 illustrates the significant interaction between time and type of pretreatment, $F(1,28)=6.29$, $p<0.05$. Caffeine given 30 minutes before d-amphetamine enhanced the d-amphetamine response, but when given 12 hours prior to d-amphetamine, caffeine attenuated the response to d-amphetamine. These results indicate that the nature of the interaction between caffeine and d-amphetamine is dependent on the pretreatment interval and can vary from enhancement of the activity effects to their attenuation.

EXPERIMENT 2

To further explore the interval parameter, we systematically varied the pretreatment interval from 0 to 13.5 hours. This study was expected to reveal whether caffeine's

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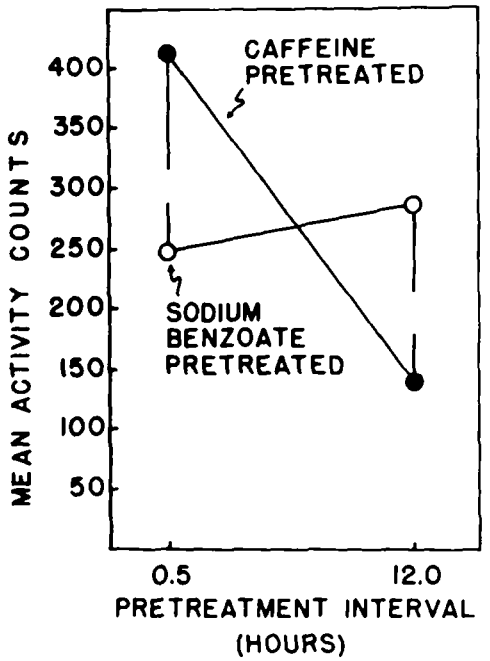

FIG. 1. Mean ± sem d-amphetamine-induced activity for rats given caffeine or sodium benzoate control injections at intervals of 0.5 or 12 hours before d-amphetamine.

enhancement of d-amphetamine follows the time course for the locomotor effects of caffeine. An earlier study [20] indicated that 89% of the stabilimeter activity during an eleven-hour period following caffeine injection occurs in the first two hours. Enhancement of d-amphetamine-induced activity may require a similar brief caffeine pretreatment interval, suggesting the summation of the locomotor effects of the two drugs.

Method

Five caffeine pretreatment groups and two sodium benzoate pretreatment groups were habituated and tested in a manner similar to that described in Experiment 1. The duration of the pretreatment intervals was the only deviation from the procedure of Experiment 1. Caffeine (30 mg/kg, IP) was given to five separate groups of 12 adult, male, hooded rats at 0, 0.5, 1.5, 4.5, and 13.5 hours before d-amphetamine. Sodium benzoate vehicle was injected at 0.5 (n=8) and 13.5 (n=7) hours prior to d-amphetamine.

Results

Timing of the caffeine pretreatment had a significant effect on d-amphetamine-induced locomotor activity, $F(5,69)=4.11$, $p<0.01$. Figure 2 illustrates the decline in the d-amphetamine response as the pretreatment interval increases. The means of the two sodium benzoate groups were only two activity counts apart, so they were collapsed into one group for statistical analysis and presentation in Fig. 2. These results are consistent with the interpretation that the augmentation of d-amphetamine activity by caffeine is related to the direct effects of caffeine on locomotion which may summate with the activation produced by d-amphetamine. At the 13.5-hour interval, caffeine pretreatment attenuated d-amphetamine-induced activity.

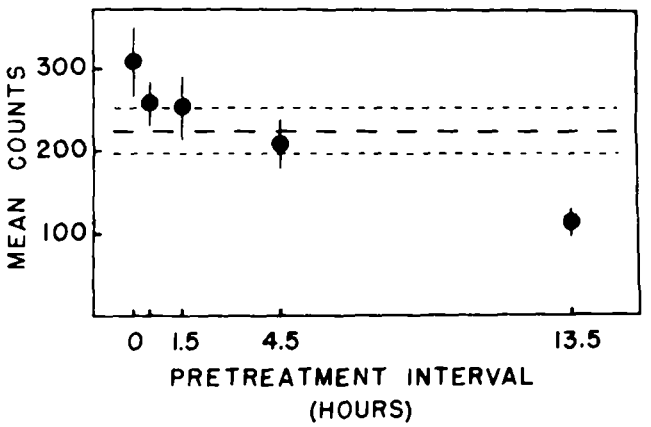

FIG. 2. Mean ± sem d-amphetamine-induced activity for rats given caffeine at one of several intervals before d-amphetamine. Dashed lines indicate mean ± sem d-amphetamine activity of sodium benzoate pretreated groups.

EXPERIMENT 3

This experiment was designed to clarify some of the parameters controlling attenuation of the d-amphetamine response by long-interval caffeine pretreatment. In addition to a range of doses, caffeine was administered twice daily for three days. This procedure allowed for the assessment of the effects of multiple caffeine treatments and doses on subsequent d-amphetamine activity.

It has been stated that little or no tolerance develops to the central stimulating effects of caffeine [10]. The series of caffeine injections in Experiment 3 provides a test for the development of tolerance to the activity effects of caffeine. A common physiological mechanism between tolerance and caffeine-induced attenuation of d-amphetamine may be suggested by the occurrence of these phenomena at similar doses.

Method

Forty-five adult male Long-Evans hooded rats were randomly assigned to one of five groups. After one week of habituation to stabilimeter activity cages each group received a series of six injections at 12-hour intervals. Four of the groups were given one of four caffeine doses (7.5, 15, 30, or 60 mg/kg, IP), and one group was given sodium benzoate control injections (60 mg/kg, IP). Twelve hours after the last caffeine or sodium benzoate each animal was injected with d-amphetamine sulfate (1.5 mg/kg, IP). Activity was recorded for a 3 hour period following injections which were given during the first and thirteenth hours of the 16-hour light phase of the daily cycle.

Results

The top panel of Fig. 3 illustrates the effect of the various doses of caffeine on locomotor activity. The first in the series of injections produced the familiar inverted U-shaped function (dose effect: $F(4,40)=21.43$, $p<0.01$). For comparison the fifth injection in the caffeine series is shown in the same figure, indicating the degree to which tolerance had developed. Significant declines in activity were found at the 15 mg, $F(1,80)=4.12$, $p<0.05$, and 30 mg doses, $F(1,80)=6.41$, $p<0.05$. The main effect due to days of treatment was also

statistically significant, $F(2,80)=13.79$, $p<0.01$. The fifth injection was compared with the first because it was the last caffeine injection that was given at the same time of day as the first. Although time of day did not significantly affect overall activity levels, $F(1,40)=2.09$, $p>0.05$, there was a significant time of day by days of treatment interaction, $F(2,80)=20.67$, $p<0.01$.

The middle panel of Fig. 3 shows that body weight was affected by caffeine treatments, $F(4,40)=7.10$, $p<0.01$. Comparison of each caffeine dose with the sodium benzoate group [22] yielded significantly lower mean daily weight gain for the 30 mg ($t=2.75$, $p<0.05$) and 60 mg doses ($t=4.79$, $p<0.01$).

The bottom panel of Fig. 3 shows the attenuation of d-amphetamine-induced activity by multiple caffeine treatments at several doses, $F(4,40)=6.76$, $p<0.01$. Only the 30 and 60 mg doses significantly depressed d-amphetamine activity when compared to sodium benzoate control group (respectively: t's=3.37 and 3.45, $p<0.01$).

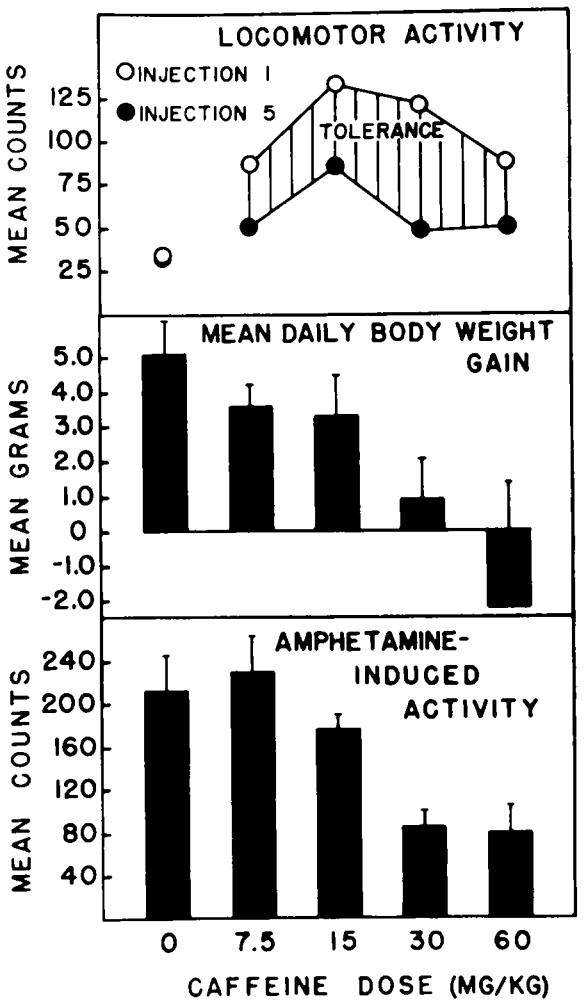

FIG. 3. Top panel. Mean activity counts for first and fifth injections of the caffeine series. Middle panel. Mean ± sem daily body weight change during 3 days of caffeine injections. Bottom panel. Mean ± sem d-amphetamine-induced activity for groups given 3 days of caffeine at doses indicated.

DISCUSSION

Experiments 1 and 2 indicate that a single caffeine treatment can alter the rat's locomotor response to d-amphetamine, producing increases or decreases in activity that depend on the timing of the pretreatment. The increase found when caffeine precedes d-amphetamine by less than an hour is similar to that previously reported as potentiation of amphetamine [13], apomorphine [14], and other catecholaminergic drugs [1, 6, 9, 16, 18].

The results of Experiment 2 suggest that the activity effects of caffeine and d-amphetamine may be additive when given within an hour of each other. The level of activity from simultaneous injection is close to that which is expected from the summation of the effects of each drug given alone. In Experiments 1 and 2, the d-amphetamine dose was selected because, under our testing conditions, it produces optimum hyperactivity. The fact that combining caffeine with d-amphetamine will increase the activity level when additional d-amphetamine does not, suggests that these drugs may act through different neurochemical mechanisms to produce locomotor activation.

Although the activity effects of both caffeine and d-amphetamine can be prevented with alpha-methyl-p-tyrosine and reinstated with l-Dopa [2,21], caffeine has been reported to sensitize catecholamine receptors [18]. In addition, recent evidence suggests that caffeine produces hyperactivity by blocking adenosine receptors which are believed to have an inhibitory effect on locomotor activity [15]. d-Amphetamine is reported to act through catecholaminergic synapses [5]. Electrophysiological recording has also yielded divergent results for caffeine and d-amphetamine. The former inhibited the firing of cells of the medial thalamus while the latter excited these cells [4]. The fact that caffeine, d-amphetamine, and other drugs [12] depend on the integrity of catecholamine neurotransmission for their activity effects may mean that a portion of the final common pathway for locomotor activity is catecholaminergic. Caffeine appears to have effects on other neurochemical systems that do not overlap with those affected by d-amphetamine.

Attenuation of d-amphetamine activity by 12-hour caffeine pretreatment reveals a new dimension to the caffeine-d-amphetamine interaction. Several lines of reasoning suggest that a toxicity reaction may be involved. The results of Experiment 3 indicate that the attenuation occurs only at the higher doses even when multiple caffeine treatments precede d-amphetamine. These effective doses are on the down side of the caffeine dose-activity curve, implying a toxic effect that limits the hyperactivity. Further support for a toxicity explanation is found in the fact that the caffeine doses effective in attenuating d-amphetamine also reduced or prevented body weight gain. In a related though nonsystematic observation, we found that the animals receiving the highest caffeine dose in Experiment 3 exhibited profuse mimetic responses by the third injection. Several "mimetic" responses have been found to occur when a rat is presented with noxious tastes [8] or the malaise of LiCl injections [7]. In the present study the rats that received the 60 mg caffeine dose exhibited gaping (repeated wide opening of the mouth) and chin rubbing (forward rubbing of the chin on the substrate).

As indicated above caffeine has been suggested to act through any of several neurochemical mechanisms including catecholamines, cyclic nucleotides [3], adenosine receptors [15], and benzodiazepine receptors [11]. As reported here the caffeine-induced attenuation of d-amphetamine activity

may be the result of compensatory changes in one or more of these biochemical systems. The fact that there was no increase in the attenuation of d-amphetamine when the caffeine pretreatment was increased from 30 to 60 mg/kg may indicate the mechanism is saturable with moderately high doses.

Tolerance to the activity effects of caffeine has been in doubt for many years. A prominent review [10] of caffeine pharmacology indicated that little or no tolerance develops to the central nervous system stimulation of caffeine. The only study available to the authors that concerns the issue of tolerance to the activity effects of caffeine was not designed to adequately demonstrate tolerance [17]. The report states that tolerance was apparently responsible for the lack of increase in wheel running activity during seven days of consuming food that contained caffeine. Although daily doses were reported, the pattern of drug intake cannot be determined from the report. Either frequent and small or infrequent and large meals could have resulted in low activity counts due to the rats getting a dose that was too low or too high. Furthermore, the absence of caffeine-induced hyperactivity at the beginning of the treatment casts doubt on whether tolerance was involved in the lack of a drug effect during the remaining 7 days of testing.

The results of Experiment 3 provide evidence that partial tolerance develops to the activity effects of moderate doses of caffeine. This decline in activity following multiple caffeine treatments does not seem to be another manifestation of the toxic reaction discussed above, because it occurred at the 15 mg dose which did not significantly affect weight gain or the d-amphetamine response.

The present study indicates the caffeine pretreatment not only affects the animal's response to additional injections of caffeine, but also the response to d-amphetamine. The nature of caffeine's influence on d-amphetamine is determined by the timing and dose of caffeine pretreatment.

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