Pharmacology Biochemistry & Behavior, Vol. 18, pp. 891–894, 1983. © Ankho International. Printed in the U.S.A.

Clonidine Potentiates Drug Induced Self-Injurious Behavior in Rats[1]

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Received 25 August 1982

MUELLER, K. AND W. L. NYHAN. *Clonidine potentiates drug induced self-injurious behavior in rats.* PHARMACOL BIOCHEM BEHAV **18**(6) 891–894, 1983.—Caffeine and amphetamine have been regarded as dramatically dissimilar drugs, both structurally and behaviorally. However, both are stimulants and (under certain conditions) both produce self-injurious behavior in rats which is potentiated by clonidine. Rats were pretreated with various doses of clonidine prior to daily administration of caffeine. In another experiment, rats which had been implanted with continuous release amphetamine pellets were injected twice daily with various doses of clonidine. Clonidine produced a high rate of self-biting when combined with subthreshold doses of caffeine. Clonidine was less effective in potentiating amphetamine induced self-biting; the highest dose tested raised the incidence from 28 to 64%. Clonidine tended to decrease the incidence of behaviors other than self-biting. Thus although caffeine and amphetamine clearly do not have identical behavioral effects, there is increasing evidence that certain of their effects are mediated by similar mechanisms.

Caffeine Amphetamine Self-biting Stereotypy Clonidine Motor activity

SELF-BITING (SB) in rodents has occasionally been reported after high doses of amphetamine [9,21] and has sometimes been assigned the maximal value on amphetamine rating scales (cf. [6,14]). SB also occurs during the intense stereotypy produced by high doses of pemoline [16], an amphetamine-like drug. However, SB can be produced in the absence of stereotypy with continuous administration of low doses of amphetamine [18] or with daily caffeine [12,18].

Amphetamine and pemoline are both well known dopaminergic agents [7,19]. Caffeine has many effects including phosphodiesterase inhibition, enhancement of central noradrenergic [2] and serotonergic activity [23] and action at central adenosine receptors [28]. Although some doses of caffeine produce hyperactivity in rats the neurochemical mechanisms of this effect are distinct from the neurochemical mechanisms of amphetamine induced hyperactivity [11]. Caffeine does not produce amphetamine-like stereotypy (repetitive sniffing, head bobbing, or licking/biting). Thus caffeine and amphetamine appear to be structurally, pharmacologically, and behaviorally distinct.

In view of these differences between caffeine and amphetamine, self-biting produced by the two drugs would also seem to be subserved by distinct neurochemical mechanisms. However, we have found that SB produced by the two drugs is similar in several respects. Latencies to SB are similar; targets of SB are similar; SB produced by continuous amphetamine is eliminated by dopamine antagonists and SB produced by daily caffeine appears to be reduced by dopamine antagonists [18]. We now report that SB produced by daily caffeine and continuous amphetamine is potentiated by clonidine, providing further evidence that caffeine and amphetamine induced SB may be subserved by similar neurochemical mechanisms.

EXPERIMENT 1

METHOD

Animals

Male Long Evans hooded rats (Simonsen, Gilroy, CA) ranging in body weight from 150 to 205 g were housed individually in polypropylene nesting boxes (54×23×20 cm). Animals were maintained on a 12 hr light/dark cycle (with diffuse low lighting during the dark cycle). Drugs were always administered during the dark cycle.

Procedure

There were 4 groups of 14 animals: 140 mg/kg caffeine, 180 mg/kg caffeine, 140 mg/kg caffeine + 0.025 mg/kg clonidine, and 140 mg/kg caffeine + 0.50 mg/kg clonidine. All injections were subcutaneous; clonidine injections were administered 30 min prior to caffeine injections. Animals were injected once daily for 10 days and were examined twice daily for self-biting. Animals which exhibited severe SB were sacrificed with an overdose of pentobarbital.

On days 1, 3, 5, 8, and 10 of drug administration, home cage behavior was recorded by observing each rat for 2 min at intervals of about 12 min for 1 hr. (Previous work indicated that most behavioral changes other than SB occurred

[1]This research was supported by NIH National Research Service Award No. AM07318, U.S. Public Health Service grant No. GM17702 from the National Institute of General Medical Sciences, and grant No. NF1377 from the March of Dimes Birth Defects Foundation.

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within the first hour after injection. At this dose caffeine does not produce hyperactivity in an open field.) This scanning system allowed for simultaneous observation of several rats. Locomotions, rears, vigorous digging/burrowing in the nesting material, and wet dog shakes were recorded as previously described [16]. To facilitate statistical analysis behavior scores were summed to arrive at daily totals.

EXPERIMENT 2

METHOD

Animals

Male Long Evans hooded rats (Charles Rivers) ranging in body weight from 170 to 240 g were housed as described in Experiment 1.

Procedure

There were 5 groups of 14 animals (unless otherwise noted): vehicle pellet (N=9), amphetamine pellet, amphetamine pellet + 0.01 mg/kg clonidine, amphetamine pellet + 0.05 mg/kg clonidine, amphetamine pellet + 0.50 mg/kg clonidine (because of unexpected findings in Experiment 1 a wider range of clonidine doses was employed in Experiment 2). Amphetamine pellets were constructed as described by Huberman [10], loaded with 46 mg amphetamine base in polyethylene glycol, and implanted under light ether anesthesia. Pellets were implanted at least 1 hr before the morning observation (see below). Clonidine was administered subcutaneously after the morning and evening observations. Animals not injected with clonidine were injected with saline.

Behavior was observed and recorded 3 times daily, morning (5 hr after lights out), afternoon (1.5 hr before lights on), and evening (4 hr after lights on). At each time behavior was observed in the home cage for 5 min and in an open field (74×66×30 cm divided into 9 areas) for 2 min. Locomotions, rears, stereotyped head movements (SHM) and licking/biting of the cage were recorded (the latter 2 behaviors were rated on a scale from 0 to 3) as previously described [16]. To facilitate statistical analysis, behavior scores were summed to arrive at daily totals. (Pilot work with a clonidine group led us to increase the height of the open field because clonidine rats tended to jump out repeatedly. A few rats from the clonidine group jumped the 30 cm wall; in such cases the animals were returned to the center area of the open field and the test was continued.)

RESULTS

As shown in Table 1 140 mg/kg caffeine was below threshold for producing SB. As little as 0.025 mg/kg clonidine produced SB and 0.5 mg/kg produced a high rate of SB ($\chi^2=11.20$, $p<0.01$ for the three 140 mg/kg groups). On days 2 and 3 of drug administration several rats exhibited renewed SB immediately after the daily clonidine injection (but before the daily caffeine injection). In the caffeine experiment 6 animals were sacrificed because of the severity of the SB (data from these animals are not presented except in describing the incidence of SB). The surviving animals exhibited body weight gains by the end of the 10 day experiment.

Other home cage behaviors of caffeine treated rats are shown in Fig. 1. (Note that locomotions and rears exhibited by 140 mg/kg caffeine rats are not significantly different from undrugged controls [16].) In most cases, clonidine pretreated rats exhibited fewer behaviors than saline pretreated rats, particularly at the higher dose of clonidine (0.50 mg/kg). Thus the potentiation of SB by clonidine was a selective effect; other behaviors tended to be reduced.

TABLE 1

INCIDENCE OF SELF-BITING (SB) OF RATS TREATED WITH DAILY CAFFEINE OR CONTINUOUS AMPHETAMINE

Caffeine			Amphetamine Pellets	
Caffeine* Dose	Clonidine Dose	SB†	Clonidine Dose	SB
140	0	0	0	4
180	0	3	0.01	3
140	0.025	4	0.05	4
140	0.500	8	0.50	0

*All doses are expressed as mg/kg body weight.
†The number of rats exhibiting SB is shown; in all groups N=14.

The two lower doses of clonidine had no effect on SB produced by the amphetamine pellets (Table 1) but 0.50 mg/kg clonidine potentiated SB. The rate of SB after 0.50 mg/kg clonidine is significantly different from the rate in the other three groups ($\chi^2=7.32$, $p<0.05$). All amphetamine pellet rats survived the experiment. Except for the 0.50 mg/kg clonidine group (which exhibited a mean weight loss of 18 g) all groups had reached their pre-experimental body weights by the conclusion of the four day study.

Persistent licking/biting and SHM were rarely exhibited by the amphetamine pellet rats. Home cage locomotions and rears tended to be highly variable, since many animals slept during the observation periods. But placing the animals in the open field revealed dramatic differences between groups (Fig. 2).

Rats implanted with amphetamine pellets were always more active in the open field than rats implanted with vehicle pellets. Of the 4 amphetamine pellet groups, the 0.50 mg/kg clonidine rats were almost always the least active; the 0.05 mg/kg clonidine group eventually became the most active. The data for rears are virtually identical to those for locomotions, and therefore are not shown.

Again clonidine exerted dissimilar effects on SB and other behaviors. Locomotions were decreased by 0.50 mg/kg clonidine but SB was increased; SB was unaffected by 0.05 mg/kg clonidine but locomotions were increased.

GENERAL DISCUSSION

Contrary to expectations, clonidine potentiated SB produced by both caffeine and continuous release amphetamine pellets. However, clonidine did appear to have a greater effect on SB produced by caffeine. Higher doses of clonidine were required to potentiate SB produced by amphetamine pellets and the magnitude of the effect was not as great. We have described previously the similarity between the targets of SB produced by amphetamine and caffeine, and the similarity between the latencies to SB [18]. Taken together these data suggest that SB produced by both amphetamine and caffeine may share, at least in part, a common neurochemical mechanism.

There have been increasing suggestions that caffeine may

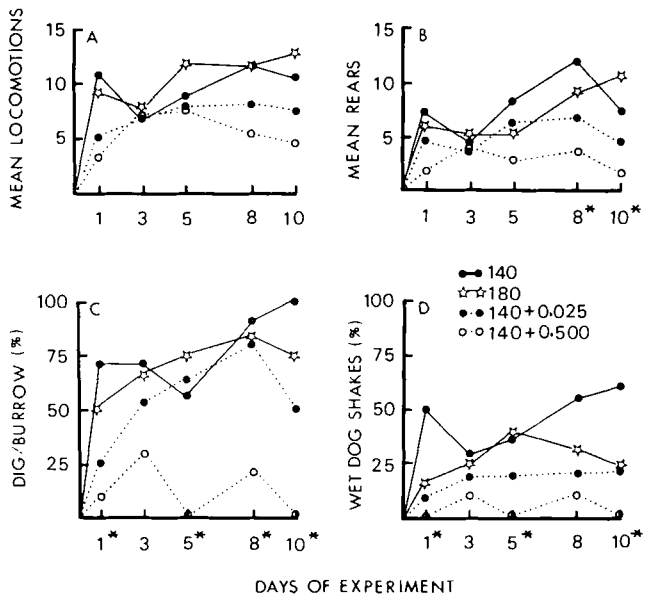

FIG. 1. Home cage behaviors of rats treated with 140 mg/kg caffeine, 180 mg/kg caffeine, 140 mg/kg caffeine + 0.025 mg/kg clonidine, or 140 mg/kg caffeine + 0.50 mg/kg clonidine. Mean locomotion counts are shown in A, mean rearing counts are shown in B, the percent of animals vigorously digging/burrowing in the bedding is shown in C, and the percent of animals exhibiting wet dog shakes is shown in D. (Locomotions and rears exhibited by 140 mg/kg caffeine rats are not significantly different from undrugged controls [17].) *Indicates statistically significant ($p<0.05$) differences between groups (ANOVA in A and B, χ^2 in C and D).

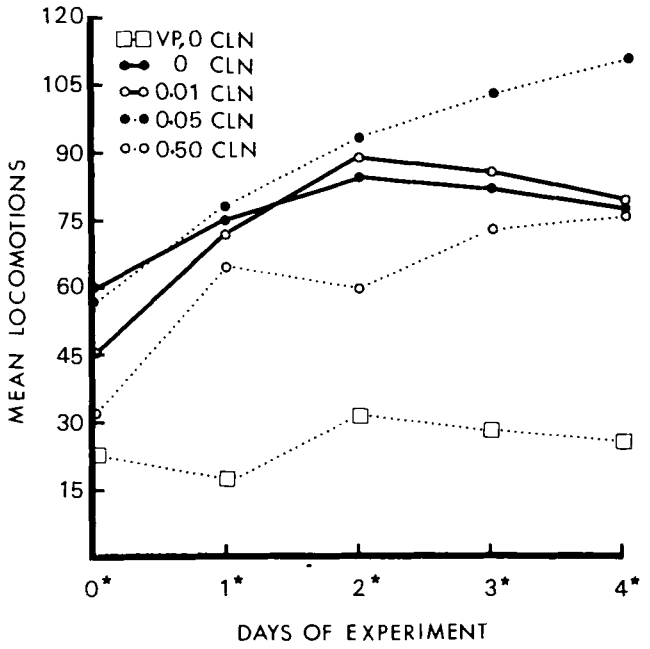

FIG. 2. Mean open field locomotion counts by rats implanted with vehicle pellets (VP) or amphetamine pellets. Clonidine (CLN) doses are shown in mg/kg. Day 0 represents the day of pellet implantation. *Indicates statistically significant differences between groups (ANOVA, $p<0.05$). The data for open field rears are very similar to those for open field locomotions.

have some effect on dopamine receptors. For example, caffeine produced rotational behavior (which was blocked by dopamine antagonists) in rats with unilateral lesions of dopamine pathways, a well known model for dopaminergic activity [27]. This effect of caffeine may be due to its actions at central adenosine receptors; there has been recent evidence that adenosine receptors modulate dopamine action in the caudate [8]. Such an interaction between purinergic and dopaminergic mechanisms would explain why caffeine and amphetamine induced self-biting appear to be so similar. Although this hypothesis is attractive, others are equally tenable at this time.

The potentiation of SB by clonidine provides additional evidence that SB produced by daily caffeine and continuous amphetamine may be subserved by similar mechanisms. However, the mechanism by which clonidine exerts this effect is unclear. Clonidine is primarily described as an αnoradrenergic agent [1, 4, 22], and there has been increasing evidence that clonidine and other noradrenergic manipulations can modify the behavioral response to amphetamine and other dopaminergic agents [3, 13, 15]. But we have recently obtained data on the effects of clonidine on behavior produced by acute amphetamine which are not easily explained by the known adrenergic effects of clonidine [17]. Clonidine has been suggested to exert some purinergic actions [25] but that finding is controversial. Because the neurochemical effects of clonidine are somewhat perplexing at this time, additional studies with other noradrenergic manipulations of SB are clearly warranted to resolve this issue.

Clonidine has been reported to produce analgesia at some doses [20]. An often suggested mechanism for SB is that the animals do not respond to pain; the loss of sensory feedback presumably leads to excessive grooming to the point of tissue damage. The point might be raised that the analgesic actions of clonidine are responsible for the potentiation of SB observed in this study. Although production of analgesia might allow a particular animal to self-bite more severely, how analgesia could increase the incidence of SB is less clear. Caffeine does not produce increased grooming in our hands. Although amphetamine pellet rats exhibit excessive grooming, grooming is unaffected by procedures which eliminate SB [18]. Animals treated with daily caffeine and continuous amphetamine are clearly responsive to both pain and tactile stimulation. The grooming/pain hypothesis seems to have arisen from the observation that dorsal rhizotomy produces SB. However, there has been evidence that the SB which accompanies dorsal rhizotomy is due to dysthesia rather than to anesthesia [5].

Surprisingly, very high doses of clonidine (50 mg/kg) have been reported to produce SB in mice [23] which is potentiated by caffeine [22]. Clonidine induced SB differs from amphetamine and caffeine induced SB; clonidine induced SB only occurs in absence of objects to bite (e.g., flood pellets, bedding) [23]. There have been no reports of clonidine induced SB in rats and we have been unable to produce this behavior in rats at up to 40 mg/kg acute clonidine or at up to 10 mg/kg of chronic clonidine. Therefore increased SB produced by far lower doses of clonidine (0.025 to 0.5 mg/kg) in this study represents a true potentiation of self-biting which warrants further investigation.

In both experiments reported here, the highest dose of clonidine (0.5 mg/kg) reduced the frequency of virtually every behavior except SB. The potentiation of SB but the

reduction of locomotions, rears, and other behaviors by clonidine suggests three hypotheses: clonidine exerts different effects on different behaviors; SB tends to exclude other behaviors (that is, the more time the animal spends SB the less time is available for other behaviors); "peripheral" effects of the high dose of clonidine (tremor, loss of muscle tone, etc.) rendered the animals unable to exhibit locomotions, rears, circling, wet dog shakes, and digging/burrowing. The first hypothesis appears to be most consistent with the data. Self-biting was not continuous in either drug group. The amphetamine pellet animals never exhibited SB in the open field, thus the problem of mutually exclusive behaviors did not always occur. Further, the amphetamine pellet/high clonidine group exhibited more open field locomotions than the vehicle pellet group. Thus an inability to exhibit particular behaviors cannot explain the data.

ACKNOWLEDGEMENTS

The authors thank Stephanie Saboda and Frank Andrijeski for technical assistance, and Stanley Garbus, Boehringer Ingelheim, for a generous supply of clonidine.

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