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Self-Injurious Behavior Produced in Rats by Daily Caffeine and Continuous Amphetamine

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MUELLER, K., S. SABODA, R. PALMOUR AND W. L. NYHAN. *Self-injurious behavior produced in rats by daily caffeine and continuous amphetamine*. PHARMAC. BIOCHEM. BEHAV. 17(4) 613–617, 1982.—Self-biting (SB) is an unusual behavioral effect of high doses of certain amphetamine-like drugs in rats. This bizarre behavior has received little attention, perhaps because the high doses of drug required and the dramatic disturbance of the animals' behavioral repertoire have raised the possibility that SB is a high dose phenomenon. However, we have found that continuous administration of very low amounts of amphetamine reliably produces SB in rats, and that this behavioral change can be very selective. We compared SB produced by continuous amphetamine to SB produced by daily caffeine; the latter has been proposed as an animal model for self-injurious behavior (SIB) in the Lesch-Nyhan syndrome. Subcutaneous silicone pellets containing amphetamine base were implanted for 4.5 days; caffeine was administered daily for 10 days. The amphetamine pellets produced the highest rate of SB (75% vs 40%) with the least toxic effects (no deaths vs three deaths). Neither drug produced stereotypy. The dopamine antagonist haloperidol was only marginally effective in controlling SB produced by daily caffeine but the dopamine antagonist pimozide (which has a longer duration of action) prevented SB by amphetamine pellet rats. Continuous release amphetamine pellets may provide an alternative to the caffeine model of SIB in humans, particularly for the Lesch-Nyhan syndrome.

Amphetamine Haloperidol Self-biting Caffeine Pimozide

SELF-BITING (SB) in rats is an unusual behavioral effect of high doses of pemoline [12,13], an amphetamine-like drug. SB generally occurs during the intense stereotypy phase of the behavioral response to the drug. Self-biting has occasionally been observed after high doses of amphetamine (cf. [16]) and in some cases has been assigned the maximal value on amphetamine rating scales [2,10]. This interesting behavior has received little attention, perhaps because it has been viewed as a "high dose" phenomenon or as a generalized extension of stereotyped gnawing. If SB could be produced in absence of other dramatic changes or toxic effects it may provide clues to the proximal mechanisms of self-injurious behavior (SIB) in humans, particularly in those cases associated with physiological disorders. We have been able to produce SB in rats without accompanying stereotypy with low doses of amphetamine. This system may be superior to the caffeine model previously proposed for SIB in humans, particularly in the Lesch-Nyhan syndrome [9].

Although there have been anecdotal reports of SB produced in rats by acute administration of amphetamine we have been unable to reproduce this phenomenon reliably. We were therefore interested to find that subcutaneously implanted silicone pellets, which release amphetamine continuously, produce SB in rats under certain circumstances. Rats implanted with these pellets entered a 3-stage behavioral pattern: constant hyperactivity and sustained motor stereotypies in the first 3 days; hypoactivity on days 3 through 5; "hallucinogenic" behaviors appearing 5 or more days after pellet implantation. This latter stage was characterized by intense grooming, wet dog shakes, spontaneous flicking movements of the forelegs and exaggerated startle responses: during this time open wound became apparent [6]. Later reports indicated that SB was also produced by removing pellets 4.5 days after implantation and challenging with a low dose of amphetamine 12 hours after pellet removal [14].

Caffeine also produces SB in rats [8,15]. Although caffeine is structurally dissimilar from amphetamine, there have been reports that caffeine, like amphetamine, may affect central dopamine neurotransmission. The increased locomotor response to caffeine was blocked by the dopamine antagonist pimozide [4], and caffeine potentiated both amphetamine and apomorphine induced stereotypy [7]. Finally, caffeine produced rotational behavior in animals with unilat-

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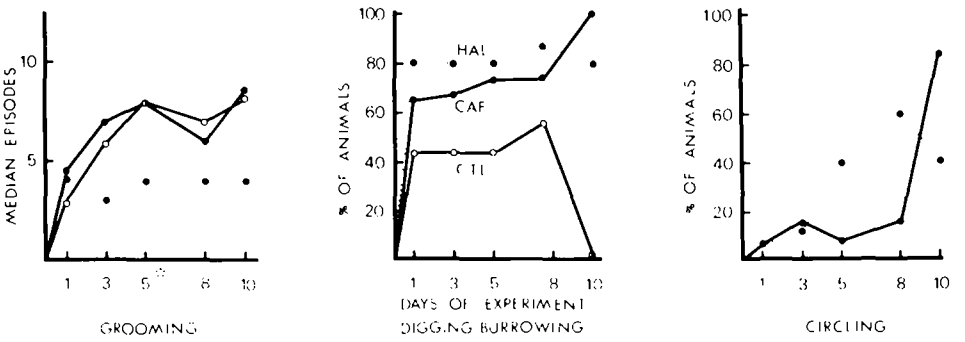

FIG. 1. Behavior during 10 days of daily caffeine (140 mg/kg/day). HAL rats were pretreated with 0.2 mg/kg haloperidol prior to administration of caffeine; CAF rats were treated with caffeine only; CTL rats were injected with vehicle only. *Denotes statistically significant ($p<0.05$) differences between groups.

eral lesions of ascending dopamine pathways, a well known model for dopaminergic activity. Rotational behavior produced by caffeine was blocked by dopamine antagonists [17]. Thus, although caffeine is dissimilar to both pemoline and amphetamine structurally, and to some extent behaviorally, we wondered whether SB produced by caffeine might involve dopaminergic mechanisms. We compared SB produced in rats by daily caffeine and continuous release amphetamine pellets and examined the effects of a dopamine antagonist on each system.

EXPERIMENT 1

METHOD

Animals

The subjects were 37 male Long Evans hooded rats (Simonsen, Gilroy, CA) ranging in body weight from 149 to 208 g at the beginning of the experiment. Animals were housed individually in polypropylene nesting boxes (54×23×20 cm) with water (in an automatic delivery system) and food available ad lib. Administration of drugs always occurred during the dark phase of an aritifical 12 hour light/dark cycle (with lights off at 3 a.m. and diffuse low lighting during the dark cycle).

Procedure

There were three experimental groups. One group (n=15) was injected with 140 mg/kg caffeine (Sigma), one group (n=15) was pretreated with 0.2 mg/kg haloperidol (McNeil) 30 min prior to administration of 140 mg/kg caffeine, and a control group (n=7) was injected with normal saline. All animals were injected once daily for 10 days; caffeine injections were subcutaneous and haloperidol injections were intraperitoneal.

Animals were examined twice daily for signs of self-injurious behavior. On days 1, 3, 5, 8, and 10 of drug administration behavior was sampled by observing each rat for 2 2-min intervals (each interval was separated by about 15 min) immediately after, 2 hr, and 4 hr after caffeine injection. This sampling procedure allowed for simultaneous observation of several animals. Locomotions, rears, grooming episodes were recorded as previously described [13]; occurrence of eating, drinking, digging/burrowing in the bedding, circling, or backwards locomotion was noted.

RESULTS

Three animals did not survive the experiment; each of the three had exhibited self-biting. Data from these animals are not included below, except in describing the incidence of self-biting. There were no deaths among animals pretreated with haloperidol or among controls. All animals gained weight during the experiment.

Among rats pretreated with haloperidol, 3 of 15 exhibited SB with 1 rat self-biting within 24 hr and the remaining 2 rats self-biting on day 10. Among rats injected with caffeine alone, 6 of 15 exhibited SB, all within 3 days of drug treatment. The most common targets of SB were the feet, with some rats biting two or three feet; lateral as well as medial digits were bitten. Occasionally SB occurred over the entire foot, with swelling and teeth marks visible up to the ankle. There were no apparent differences in other aspects of behavior between rats that self-bit and rats that did not.

Because there were no differences between the three groups with regard to the temporal distribution of behaviors on each observation day, behavior scores for each of the three sampling periods were summed to arrive at a daily total. Mean locomotions during the 10 days were similar (with all groups generally exhibiting between 5 and 10 locomotion counts per day). Likewise, the amount of rearing did not differentiate between groups (with animals usually exhibiting between four and eight rearing counts).

Thus caffeine did not produce amphetamine-like hyperactivity. However, caffeine treated rats did expend more energy than controls by digging/burrowing and by circling. Tight circling gradually changed from a low to high frequency behavior during the experiment (Fig. 1). By day 8, circling was often rapid and frenzied. Digging/burrowing was always a high frequency behavior for caffeine treated rats. By day 5 digging/burrowing had become energetic, with most rats repetitively relocating large amounts of bedding. Backwards locomotion was maximal on day 19, with about 30% of caffeine treated rats exhibiting this unusual behavior.

As indicated above, pretreatment with haloperidol had little effect on most behaviors. However, the number of grooming episodes was consistently reduced by haloperidol (Fig. 1). This reduction was statistically significant

(Kruskal-Wallis ANOVA, $H(2)=7.96$, $p<0.05$). (Medians and nonparametric techniques are reported as the number of grooming episodes was not deemed to fulfill the requirements of a ratio scale.)

DISCUSSION

As others have demonstrated [9,15] daily caffeine produced SB in rats which could be severe. Pretreatment with haloperidol protected animals from the toxic effects of caffeine and reduced the incidence of self-biting. Although the effect of haloperidol was not as dramatic as one would expect for a behavior mediated primarily by dopaminergic mechanisms, there were suggestions that haloperidol may have had some effect on SB. The delayed SB exhibited by 2 of the 3 haloperidol self-biters is very unusual and considered in conjunction with the reduction of SB suggests that further research into the dopaminergic nature of caffeine induced SB is justified.

Most animals appeared to develop tolerance to the ability of caffeine to produce SB. Except for the two haloperidol pretreated rats mentioned above, in this and subsequent studies virtually all SB occurs within three days of caffeine administration. In contrast, other unusual behaviors, such as circling and backwards locomotion, occurred most often in later portions of the experiment. Digging/burrowing was a high frequency behavior throughout the experiment, but appeared to increase in intensity as the drug administration continued. The different temporal aspects of these behaviors suggest that backwards locomotion, circling, and digging/burrowing may be mediated by different neurochemical mechanisms than SB.

EXPERIMENT 2

METHOD

Animals

The subjects were 34 male Long Evans hooded rats, ranging in body weight from 174 to 223 g at the beginning of the experiment. Animals were housed as described in Experiment 1.

Procedure

Briefly, the procedure was as follows. Subcutaneous pellets were implanted at 9 a.m. (day 0) and 4.5 days later pellets were removed (7 p.m.). The following morning (9 a.m.) animals were challenged with 3 mg/kg amphetamine sulfate (Sigma) intraperitoneally.

Silicone pellets were constructed as previously described [6] and contained vehicle or 43±3.6 mg amphetamine base suspended in polyethylene glycol. Approximately 54% of the amphetamine was released from the pellet (as determined by assay of pellet contents) during the 4.5 days of implantation. Pellets were implanted and removed under light ether anesthesia.

Animals were observed twice daily, at 8 a.m. and 6 p.m. At each observation period behavior was recorded in the home cage for 5 min and in an open field (74×66×15 cm divided into 9 areas) for 2 min. During the amphetamine challenge home cage behavior was recorded for 2 min at 2-min intervals for 1 hr. Locomotions, rears, and grooming episodes were recorded as previously described. Stereotyped head movements and licking/biting of the cage were rated on a scale from 0 to 3.

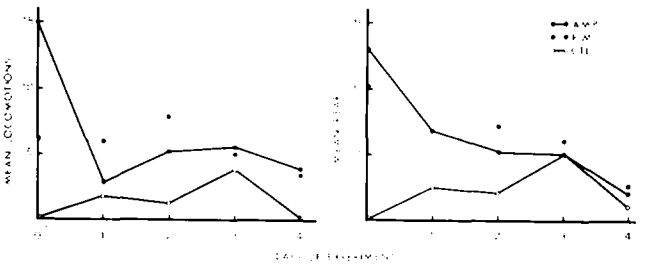

FIG. 2. Home cage locomotions and rears during 4.5 days of amphetamine pellet implantation. CTL rats were implanted with a pellet containing vehicle only; PIM rats were implanted with amphetamine pellets but received daily pimozide injections; AMP rats were implanted with amphetamine pellets and received vehicle injections. *Denotes statistically significant differences between groups.

The rats were divided into 3 groups: 10 rats were implanted with control pellets (vehicle only); 24 rats were implanted with amphetamine pellets. Of the latter, 12 were injected intraperitoneally with 1.5 mg/kg pimozide (Janssen) (pimozide has a longer half-life than haloperidol, and therefore seemed more suitable than haloperidol for use with continuous amphetamine administration). Because pimozide is a relatively slow acting drug, the first injection was given at 6 p.m. the evening prior to pellet implantation; the animals were also injected 1 hr before surgery. On following days pimozide was administered after the morning observation; no pimozide was administered on the day of pellet removal or on the day of the amphetamine challenge.

Brain amphetamine levels were determined by the method of Axelrod [1] using an additional six animals. Amphetamine determinations were made at 24 and 48 hr after pellet implantation.

RESULTS

Brain amphetamine levels ranged from 1.30 to 1.66 and 1.22 to 1.50 μg/g brain 24 and 48 hr, respectively, after pellet implantation. All animals survived the experiment and maintained steady body weights. No self-biting was observed in either the control or pimozide treated rats. Of the 12 amphetamine only rats, 9 exhibited SB, 7 within the first 48 hr after pellet implantation (range=10 to 82 hr, Mdn=34 hr). Self-biting usually continued for about two days although one rat self-bit for five days. The most common targets of self-biting were again the feet, with lateral as well as medial surfaces bitten. One rat bit his thorax; another bit his tail. The severity of SB ranged from fur removal to amputation of digits or removal of large areas of skin. No self-biting was recorded during the amphetamine challenge which followed pellet removal.

Behavior scores for both the morning and evening observation periods were summed to arrive at a daily total. During the 4.5 days the pellets remained in place, home cage observations revealed statistically significant differences in locomotions and rears only on the day of pellet implantation (see Fig. 2). At this time there were significant increases in locomotions, $F(2,31)=29.22$, $p<0.01$, and rears, $F(2,31)=5.15$, $0<0.025$, in both amphetamine pellet groups

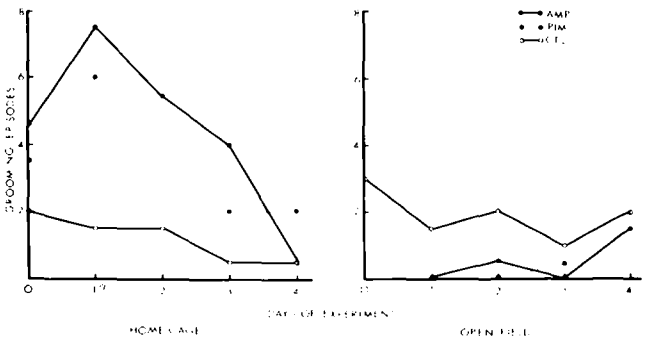

FIG. 3. Median grooming episodes during 4.5 days of amphetamine pellet implantation. The legend is the same as in Fig. 2.

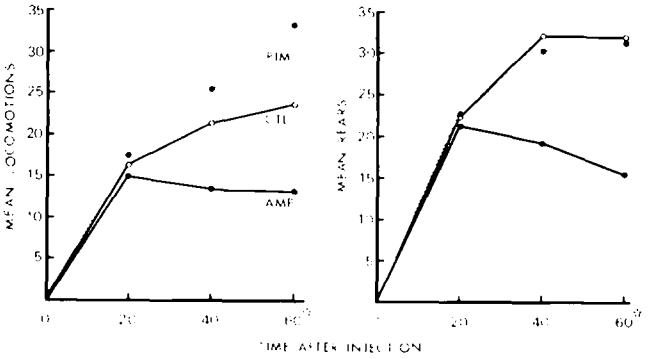

FIG. 4. Locomotions and rears during an amphetamine challenge (14 hr after pellet removal). The legend is the same as in Fig. 2.

as compared with controls. There was also a consistent increase in grooming in the pellet rats over controls during the first three days of the experiment (see Fig. 3). These increases were statistically significant only on the day following pellet implantation, $H(2)=5.99$, $p<0.05$. Other behaviors, such as licking/biting and stereotyped head movements were seldom observed.

In the open field the amphetamine pellet rats exhibited increased locomotions during the first two days but these differences were not significant. (Locomotions generally ranged from 20 to 30 lines crossed.) There were no significant differences in the mean number of rears (with all 3 groups exhibiting about 12 to 18 rears). On the day of pellet implantation, there was a statistically significant reduction in the number of grooming episodes by the pellet groups, $H(2)=11.37$, $p<0.01$. These differences became less obvious on following days (see Fig. 3). Thus the amphetamine pellets produced highly selective behavioral changes.

Behavior scores for the amphetamine challenge were obtained by summing the 2-min observations into 3 20-min time periods (20, 40, and 60 min). There were decreases in the mean number of locomotions, $F(2,32)=7.37$, $p<0.025$, and rears $F(2,31)=4.65$, $p<0.05$, in the amphetamine only group as compared to the pimozide and control groups (see Fig. 4). Median grooming episodes were consistently different between all three groups, $H(2)=12.18$, $p<0.01$. Amphetamine only rats exhibited the most grooming episodes (Mdn=35.5), followed by pimozide rats (Mdn=22); controls exhibited the fewest grooming episodes (Mdn=11.5).

DISCUSSION

Subcutaneously implanted silicone pellets, which release amphetamine continuously, produced SB in rats without producing other dramatic behavioral changes. SB was eliminated by pimozide, a dopamine antagonist. This drug also prevented the subsensitivity to an amphetamine challenge usually exhibited by amphetamine pellet rats [6,14]. The effectiveness of pimozide in controlling SB and restoring responsiveness to the amphetamine challenge supports the hypothesis that dopaminergic mechanisms are intimately involved in SB produced by amphetamine pellets.

Brain amphetamine levels were very low during pellet implantation and amphetamine did not appear to accumulate as time progressed. Amphetamine levels were lower than those reported after single injection of 2 mg/kg amphetamine sulfate and were slightly lower than those reported previously in pellet rats [6]. Thus drug induced SB is not necessarily a high dose phenomenon.

The behavior of our amphetamine pellet rats differed from that of previous reports in two major respects. We observed SB during the first 48 hr of pellet implantation rather than during the challenge, and we observed little evidence of sustained stereotypic behavior. Such obvious examples of stereotypy as headweaving, continuous sniffing or repetitive licking of the cage were virtually never observed. Furthermore, open field activity by pellet rats was inconsistent with stereotyped behavior. That is, rats exhibiting stereotypy are hypoactive in both the home cage and open field [12]. Although many variables could account for different findings in different laboratories, we believe that the lower amount of amphetamine in our pellets was the crucial variable in accounting for these differences. Pilot work has indicated that altering the amount of amphetamine in the pellet by as little as 10 mg can alter both the appearance and duration of hyperactivity and alter the incidence of self-biting (data not shown).

GENERAL DISCUSSION

Both daily caffeine and amphetamine pellets produced SB in rats. Caffeine was less effective than amphetamine in producing SB, but was also more toxic. Both drugs produced SB without producing stereotyped behavior. The dopamine antagonist pimozide prevented SB by amphetamine pellet rats but the dopamine antagonist haloperidol was only marginally effective in controlling SB produced by daily caffeine.

We have previously described self-biting produced in rats by pemoline [12,13], a long lasting amphetamine-like drug which is thought to produce its behavioral effects by selectively releasing and inhibiting reuptake of central dopamine [5,11]. Pemoline induced SB is eliminated by both haloperidol and pimozide [13]. We believe that pemoline and amphetamine pellets both produce SB by the same mechanism. Both drugs have major effects on central dopaminergic neurotransmission. Both drugs produce SB which is eliminated by dopamine antagonists. Acute administration of appropriate doses of both drugs produced classic amphetamine-like stereotyped behavior.

There were, however, two differences between SB produced by amphetamine pellets and by pemoline.

Pemoline induced SB was accompanied by dramatic stereotyped behavior and the targets of SB were highly localized (usually the medial surface of the foreleg or the medial digits). In contrast, amphetamine stereotypy was absent in amphetamine pellet rats, and the targets of self-biting were more varied. The highly localized targets of the pemoline treated rats may have been secondary to the long lasting stereotypy; stereotyped behavior by definition implies a restriction of the behavioral repertoire and this may apply to SB as well. Remembering that acute amphetamine produces dramatic stereotyped behavior, we hypothesize that continuous administration of lower doses of pemoline would produce SB in rats without stereotyped behavior, much the same as continuous amphetamine.

Whether caffeine induced SB shares a common mechanism with pemoline and amphetamine induced self-biting is unclear. The temporal aspects of SB were similar in rats treated with daily caffeine and amphetamine pellets. That is, most SB appeared within 48 hr of drug administration. Targets of SB were also similar, and neither drug produced classic stereotypy. Caffeine, did however, produce behaviors which were not seen after pemoline or amphetamine pellets, but as discussed previously, these may not share the same mechanism as SB. However, haloperidol was not as effective in reducing SB as we would have expected if dopaminergic mechanisms were the primary ones involved in caffeine induced SB.

Both caffeine [9] and pemoline [13] induced SB have been offered as animal models of SIB in humans, particularly for the Lesch-Nyhan syndrome. At this point we believe that SB produced by continuous release amphetamine pellets is superior to both the caffeine and pemoline models for several reasons. Amphetamine pellets produce more specific behavioral effects at lower doses. In addition, after several days of implantation these pellets apparently exert selective toxic effects at dopamine terminals in the caudate [3]. A recent report describes loss of dopamine in the caudate of Lesch-Nyhan patients which is consistent with reduced dopaminergic terminals [8]. Thus rats implanted with continuous release amphetamine pellets share both behavioral and physiological findings with Lesch-Nyhan patients.

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