BEHAVIORAL AND NEURAL BIOLOGY **50,** 37–45 (1988)

Excessive Grooming Induced by the Administration of Codeine and Morphine

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Previous studies have shown that peripheral injections of the codeinone RX 336-M can induce excessive grooming in rats in an age-dependent fashion. The present experiment demonstrates that codeine, itself, also induces excessive grooming but with apparent equal effectiveness at all ages tested. Because of the structural similarity between codeine and morphine, the effects of intraperitoneally administered morphine were examined as well. Morphine was found to produce a temporal course of excessive grooming quite different from that produced by codeine. Morphine did not affect grooming in the first half (30 min) of the observation period, but accentuated it, briefly, for the next 30–45 min. Intraventricular administration of codeine at doses of 0.3 or 1.0 μg/3 μl had no effect on grooming in animals previously shown to demonstrate excessive grooming in response to either dose.

In 1982 Gmerek and Cowan demonstrated that the peripheral injection of an experimental codeinone, RX 336-M, could induce substantial excessive grooming. Before this, the only reports of excessive grooming induced by peripheral injections had been of those occurring after the administration of low doses of morphine (Ayhan & Randrup, 1973; Fog, 1970). In their study, Gmerek and Cowan noted that the administration of the codeinone was especially effective in inducing excessive grooming in young rats, i.e., those weighing less than 150 g (estimated to be 40–45 days of age). These authors also presented evidence that stimulation of the traditional high-affinity μ-opiate receptor was not the cause of the codeinone-induced excessive grooming, since both it and intraventricular

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0163-1047/88 $3.00

(icv) ACTH-induced excessive grooming were antagonized by pretreatment with morphine (Gmerek & Cowan, 1983).

The observations of Gmerek and Cowan led Isaacson, Hardy, and Hannigan (1987) to conduct a more comprehensive study of age–drug relationships. They found a decreasing ability of RX 336-M to induce excessive grooming and "wet-dog shakes" in the 20- to 50-day postnatal period. By 60 days of age, the animals no longer exhibited excessive grooming in response to the drug.

In this paper we sought to determine if the peripheral administration of codeine, itself, would induce excessive grooming or whether the response was limited to the substituted analog used by Cowan and his associates (Cowan, 1981; Gmerek & Cowan, 1982, 1983).

EXPE IMENT 1

Method

Three age groups of male Wistar rats of an inbred strain (TNO, Zeist, NL) were used in this study. The first group of twelve 24-day-old animals was tested 2 days after weaning and their individual housing. The second group consisted of 8 male rats 45 days of age, and the third group was of 10 adult male rats 6–7 months of age. The latter two groups of animals had been housed two per cage since their weaning at 23 days. Codeine was given as the hydrochloride in saline at a dose of 3.3 mg/kg/ml.This provided each animal with about 3.0 mg/kg body wt of the free base of the drug. Half of the animals were injected intraperitoneally (ip) with codeine and the other half of the animals received saline alone. Fifteen minutes later, the animals were placed in individual observation chambers and observed for the next 60 min as described in Gispen, Wiegant, Greven, and de Weid (1975). Grooming scores were tabulated for each animal over every 5-min interval and over the entire observation period.

Results

The median amounts of grooming exhibited over the hour of observation by the animals of the different ages are shown in Fig. 1.

All animals given codeine groomed more than twice as much as those given saline at all ages. In the youngest group there was no overlap in the distributions of scores. The lowest grooming score obtained for any codeine-treated animal was 101 in the 60-min period. The highest score obtained by any saline-treated animal was 51. The saline-treated animals essentially stopped grooming by the fifth 5-min interval, that is, after the animals had been in the apparatus about 45 min. In contrast, the grooming scores of the codeine group were essentially the same throughout the grooming period.

With the 45-day-old subjects a similar pattern was observed, although the amount of codeine-induced grooming was somewhat, but not signif-

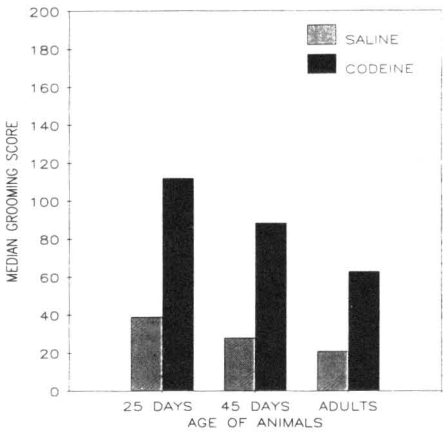

FIG. 1. Median grooming scores of independent groups of rats of three different ages injected (ip) with 3.3 mg/kg/ml codeine or saline.

icantly, less (Fig. 1). In this age group, the highest score of any saline-treated animal was the same as the lowest score of any animal in the codeine group. With this single exception there was no overlap in the distributions. Similar to the younger animals, the saline-treated animals stopped grooming early half-way through the observation period; the codeine-treated animals did not.

There was no overlap in the scores of the codeine-treated adult animals and those of the saline-treated adult animals. After the first 5-min interval, the saline-injected animals exhibited little or no grooming. The codeine-treated animals, on the other hand, showed a rather flat distribution of grooming scores across the twelve 5-min intervals, with 9 out of the 12 intervals being between 4.2 and 9.6.

Discussion

The results of this experiment indicate that the ability to elicit excessive grooming subsequent to peripheral administration of the drug is not limited to the artifical codeinone RX 336-M, but can also be produced by codeine itself. Furthermore, effectiveness of RX 336-M is eliciting grooming was age-related whereas with codeine, itself, administration at all ages induced excessive grooming. It is possible, of course, that grooming might have been induced in adults if a larger dose of RX 336-M had been used. Ideally it would have been useful to compare the elicitation of excessive grooming by the artificial codeinone with codeine over several doses, at least. Unfortunately, it was not possible to make such direct comparisons because we were unable to obtain additional RX 336-M from the manufacturer, Reckitt and Colman.

Because of the apparently contradictory results obtained by others

with peripherally injected morphine (as noted above) and because at least some amount of codeine is converted into morphine after peripheral administration, we tested other animals with a dose of morphine that would be comparable to that of codeine, i.e., approximately 3.0 mg/kg free base. This dose was well below that found to produce lethargy and/or catalepsy (Ayhan & Randrup, 1973).

EXPERIMENT 2

Method

The subjects were 12 male and 12 female albino Wistar rats 25 days of age. All animals were group-housed until the time of the first test. After that test they were then individually housed.

The animals were tested twice, 2 days apart, once after ip saline injection, and again, 2 days later, following the ip administration of 3.3 mg/kg morphine hydrochloride (3.0 mg/kg free base) in saline. On a nonsystematic basis, half of the animals received saline on the first test day and the other half received morphine. On the second test day, those animals given saline on the first day received morphine, and those previously given morphine received saline. The volume of saline, or saline and morphine, given the animals was 0.01 ml per gram body wt.

Immediately after the ip injections the animals were placed in the same individual observation chambers used in the first experiment. The animals were tested in two "squads" of 12 animals each on both days. For half of the animals, the observation time was 75 min, for the other half it was 60 min. Since there were no differences between males and females, the data from animals of the sexes were combined. Because of problems with the computer on which the scoring was recorded, the number of rats contributing to the first six 5-min intervals is less than 24, i.e., 18, the number contributing to intervals 7 through 12 is 24. The number of rats contributing to the data from intervals 13 through 15 is 12, since only half of the animals were tested this long.

Results

During the first 30 min, the morphine-treated animals exhibited levels of grooming that were not significantly different from those of the saline-treated group. However, every morphine-treated animal exhibited as much or more grooming in the second 30-min period than in the first, while only 5 of the 12 saline-treated animals increased grooming in the second half of the hour-long observation period relative to the first 30 min. Therefore, the effect of morphine on grooming was noted only in the last 30 min of the standard hour-long observation period. As noted under Methods, half of the animals received saline and the other half morphine on the first day. On the second day, the treatments were reversed. Unexpectedly, the animals exhibited somewhat different be-

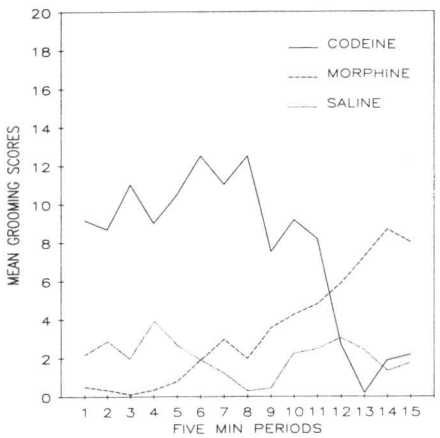

FIG. 2. The time course of grooming elicited by morphine or saline over the 75-min period. The time course of grooming exhibited by animals of a comparable age injected with codeine (from Fig. 1) is also exhibited for comparison purposes.

haviors on the 2 test days after each treatment. The saline-treated animals exhibited greater variability in the grooming on the second test day, after having receiving morphine on the first, than the group receiving saline on the first test day ($F(11, 1) = 3.75$, $p < .025$). This occurred despite similar mean grooming scores: Saline group Day 1 = 8.17; Saline group Day 2 = 8.67). The animals tested following morphine on Test Day 2 had higher mean grooming scores (mean = 38,4) than those tested on Day 1 after morphine (mean = 18.2). However, on each of the test days the animals receiving morphine groomed significantly more during the final 30 min of the observation period than did the saline-treated animals (Wilcoxon tests, $p < .01$).

For those animals observed for an additional 15 min (min 60–75), the saline-treated animals showed no change in the amount of grooming exhibited relative to the prior 15-min, a negligible, small amount.

The amounts of grooming exhibited in this "extra" 15 min of observation varied between the 2 test days. The saline-treated rats exhibited more grooming on the first test day (median = 6.5) than the second (median = 0). The morphine-treated rats did the opposite: median on Test Day 1 = 17.5; median on Test Day 2 = 26.0. However, on both test days the differences between the saline- and morphine-treated groups was significant (Test Day 1, Wilcoxon test $p < .05$; Test Day 2, Wilcoxon test, $p < .01$).

While the differences occurring between test days and prior treatments make the results seem complicated, the overall pattern of the results are clear. To demonstrate this, Fig. 2 was prepared to show the changes in the mean performances of the morphine- and saline-treated groups although

the reader should be aware that (1) in making this figure the points do not reflect the numerical data used in the statistical analyses and (2) data from both test days have been combined. The same animals are represented in the saline- and the morphine- treatment curves. Also, in this figure the data from the animals receiving ip codeine in Experiment 1 are also presented. This ip codeine curve is presented only for illustrative purposes, to show the temporal and quantitative differences between morphine- and codeine-induced excessive grooming.

Discussion

The present data illustrate that the effects of peripherally administered codeine and morphine include the elicitation of excessive grooming, although the time course of this excessive grooming is quite different for the two drugs. The peripheral administration of morphine produces a pattern of excessive grooming that bears a marked resemblance to the effect of icv morphine in which excessive grooming is found only after the first 30 min of the observation period (Isaacson, Hannigan, Brakkee, & Gispen, 1983). Morphine-induced grooming becomes increased at approximately the time that the codeine effects are disappearing.

Despite the demonstration of codeine-induced excessive grooming, the site(s) of action of either RX 336-M or codeine is not known. As a first step toward such understanding, we attempted to determine if codeine acted directly on central mechanisms that can be reached by intraventricular injection. *A priori* it is not necessary for the central nervous system to be involved since it has been shown that histaminergic drugs with largely, if not exclusively, peripheral actions can influence excessive grooming (O'Callaghan, Horowitz, & Isaacson, 1982). Therefore, we undertook a further experiment in which codeine at two dosage levels (0.3 and 1.0 μg) was administered icv to eight adult Wistar albino rats (TNO, Zeist, NL) and their behavior was observed over the following 55 min.

Plastic cannulae were implanted into the intraventricular foramen according to methods described elsewhere (Brakkee, Wiegant, & Gispen, 1979; Gispen et al., 1975). Following a 1-week postsurgical recovery period, the animals were first injected icv ACTH 1-24 (0.3 μg/3μl) to determine if the cannulae were effectively placed for the induction of grooming (Brakkee et al., 1979). Only those animals exhibiting excessive grooming subsequent to icv ACTH were used in the tests. The animals were injected icv with codeine, at two doses (0.3 μg/3μl; 1.0 μg/3 μl) on separate occasions. The administration of codeine into the intraventricular foramen failed to produce any enhancement of grooming beyond that usually found after saline administration during the observation period (data not shown).

Supporting the earlier conclusions of Gmerek and Cowan, the codeine-induced effects do not seem to be mediated by the high-affinity opiate

receptor affected by morphine. While morphine antagonized both the effects of RX 336-M and ACTH in the earlier studies of Cowan and his associates, morphine did not reduce "spontaneous" excessive grooming produced as an after-effect of handling during the early portion of the observation period. However, it is likely that a morphine-induced suppression would be statistically significant if only a restricted time interval were to be evaluated. For example, there appeared to be a depression of grooming during the first 15 min of the observation period (as seen in Fig. 2) that would have been significant relative to the grooming following saline had this been a period selected for analysis. However, this sort of *post hoc* selection of intervals to be evaluated could be misleading, although it does provide a suggestion for future research.

In general our results are in accord with the observations of Fog (1970) and Ayhan and Randrup (1973) in which the animals treated with 1 or 2 mg/kg of morphine began their excessive grooming 45–60 min after injection. The maximal amount of grooming was found about 75 min after injection that diminished rapidly to control levels by 90 min post-injection (unpublished observations). Thus while morphine can induce excessive grooming it is slow in onset and of short duration. The excessive grooming produced by morphine is obviously different in its duration and time course from the excessive grooming induced by codeine. In a sense, the two types of grooming are virtually temporal mirror images of each other.

While codeine has been little studied and is usually considered in the family of morphine-like drugs (e.g., Gilman, Goodman, Rall, & Murad, 1985), its effects are greater than those of morphine on a low-affinity μ-receptor thought to be associated, primarily, with cough suppression. This low-affinity receptor exists at close to adult levels early in life, while the high-affinity (morphine) receptor is scarcely present (Clendeninn, Petraitis, & Simon, 1976). This suggests that there is a low-affinity μ-receptor, presumably predominant in brain stem areas, that is primarily responsible for the excessive grooming that is accessible to codeine into adulthood but is more easily affected by RX 336-M early in life.

Even though the injection of codeine into the ventricular system at the two dosages used in this study failed to induce excessive grooming, it is not possible to conclude that the drug is without central effects. It is possible that our choice of dosages was inappropriate or that the site of action in the brain is not easily accessed by the cerebrospinal fluid. On the other hand, it is also possible that the excessive grooming we observed is produced by a metabolite of codeine formed in the periphery which then passes into the brain to produce its effects. However, the main metabolite of codeine is a consequence of conjugation with glucuronic acid at hydroxyl groups to form a 6-*O*-glucuronide or an *N*-methylation

to norcodeine in humans. Only a small percentage of codeine is *O*-demethylated to morphine (Muhtadi & Hassan, 1981).

The recent discovery of the presence and the synthesis of endogenous codeine and morphine by various tissues and organ systems, including the brain, in several animal species indicates the possibility that the natural ligand for the μ-receptor is morphine, itself (Donnerer, Oka, Brossi, Rice & Spector, 1986). Codeine and morphine appear to have independence from each other in terms of localization and conjugations, at least, although it is possible that codeine serves in somewhat the same fashion as dopamine in regard to the catecholamines, namely as a precursor to norepinephrine and as a biologically active agent in its own right. A functional significance of endogenous codeine and morphine is suggested by their presence in synaptosomal fractions and by the enhancement of endogenous morphine levels in the spinal cords of arthritic rats (Donnerer, Cardinale, Coffey, Lisek, Jardine, & Spector, 1987). Therefore, it is likely that the results of the present study are not only relevant to pharmacologic interventions but may be useful as indicators of alterations occurring in the physiological actions of morphine and codeine as naturally present, biologically active substances.

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