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RAPID COMMUNICATION

Effect of Stress on Oral Morphine and Fentanyl Self-Administration in Rats

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SHAHAM, Y., K. ALVARES, S. M. NESPOR AND N. E. GRUNBERG. *Effect of stress on oral morphine and fentanyl self-administration in rats.* PHARMACOL BIOCHEM BEHAV 41(3) 615–619, 1992.—The effect of immobilization stress (15 min/day) or no stress on oral morphine (0.25–0.5 mg/ml) or fentanyl (5–20 μg/ml) self-administration was examined in rats. Animals had access to a morphine or fentanyl solution for 4 days, followed by a single-choice day of access to the opioid solution and a separate water bottle. This 5-day cycle was repeated five times for 7 h/day in home cages. Morphine consumption and preference were assessed for an additional 30 days (i.e., six more cycles) in a subgroup of subjects. Plasma corticosterone levels in the stress groups indicated that the stress manipulation was effective. Over the course of the experiment, animals in the stress groups significantly increased their preference for the opioid solutions during choice days compared to nonstress controls. Morphine preference after 55 days was twice as high in the stress group (70% morphine/30% water) in comparison to controls (34% morphine/66% water). These results indicate that stress increases oral opioid self-administration in rats. Future directions and the implications of this work are discussed.

Stress Morphine Fentanyl Self-administration

EPIDEMIOLOGICAL and clinical data indicate that stress is positively related to use and abuse of opioids and other addictive drugs (17,25,28). Unfortunately, these studies are correlational, rely on retrospective self-reports of stress, and frequently rely on self-reports of illegal opiate use as well. As a result of these methodological limitations, no firm conclusion concerning a causal link can be drawn and mechanisms underlying the stress–drug use relationship cannot be clearly established (10,12,23).

Animal models could be used to directly examine effects of stress on opioid use and, in fact, some reports suggest that aversive environmental conditions and opioid self-administration are related. For example, several drug self-administration studies reported that social isolation (1,3,11) or food deprivation (5) are associated with increased opioid intake and preference. It is unclear, however, whether these environmental conditions are stressors because none of the archived studies report changes in objective indices of the stress response [e.g., plasma catecholamine (18) or corticosteroid levels (16,20,24)]. Further, other studies reported that, in rats, neither isolation (7,9,13) nor food deprivation (6) affect biological parameters of stress such as plasma norepinephrine, ACTH, corticosterone, or blood pressure. In addition, it has been hypothesized that increased drug self-administration under conditions of food deprivation may be related to variables other than stress, such as the effect of drugs on the hunger drive (10), or may illustrate a more general phenomenon, "reinforcement interaction," whereby decreased availability of one reinforcer increases responding maintained by another (5). Therefore, there are no clear reports indicating that stress results in increased opioid consumption in animals, despite the potential value of such models to investigate the presumed relationship between stress and opioid abuse.

The present experiment examined the effect of 15 min/day immobilization stress, a standard stressor that affects a variety of biological parameters associated with the stress response (16,18,20), on consumption and preference of morphine or fentanyl (a synthetic opioid). The experiment used a modified version of an animal model of oral morphine self-administration reported by Stolerman and Kumar (27). It was hypothesized that immobilization stress would cause an increase in opioid self-administration under controlled laboratory conditions.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

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METHOD

Subjects

The subjects were 25 male Sprague-Dawley rats (Taconic Farm, Germantown, NJ) and 25 male Wistar rats (Charles River, Wilmington, MA). All rats were 8–9 weeks old and weighed 250–300 g at the beginning of the experiment. Animals were individually housed in polypropylene shoebox cages (35.6 × 15.2 × 20.3 cm) at a temperature of 23°C, relative humidity of 50%, and light–dark cycles of 12 h each. Food (Purina rat chow) was continuously available.

Drugs

Morphine-sulfate (Mallinckrodt) in concentrations of either 0.25 or 0.5 mg/ml dissolved in tapwater or fentanyl-citrate (Sigma) in concentrations of either 5, 10, or 20 μg/ml dissolved in tapwater were used (drug weights are expressed as their salts). The solutions were presented in inverted 500-ml Plexiglas water bottles with rubber stoppers and nonleaking metal spouts. The opioid antagonist naloxone (Dupont) was used to precipitate the withdrawal syndrome.

Procedure

Animals were divided into four treatment groups: control/morphine (C/M, $n = 15$); stress/morphine (S/M, $n = 10$); control/fentanyl (C/F, $n = 15$); and stress/fentanyl (S/F, $n = 10$). Animals had access to morphine or fentanyl solutions every day, and also had access to water in separate bottles every fifth day. The oral morphine, fentanyl, or water were made available for 7 h a day (between 10 a.m. and 5 p.m.). Baseline water consumption was measured for 3 days prior to the start of the stress manipulation. For the morphine groups, the first choice day was between a 0.5-mg/ml morphine solution and water after 4 days of access to only a 0.25-mg/ml morphine solution. For the fentanyl groups, a 5-μg/ml fentanyl solution was provided for the first 4 forced-consumption days. A 10-μg/ml fentanyl solution was provided on the first choice day and for the subsequent four cycles of forced consumption and choice. A 20-μg/ml fentanyl solution was provided during the last forced-consumption days and the last choice day. All animals participated in the study for 5 choice days (25 days). Morphine consumption and preference in subgroups of the morphine animals (five control and four stress animals) were assessed for a longer time period (i.e., 11 choice days or 55 days). Naloxone-HCl (1 mg/kg) was injected IP on day 23 of the experiment and symptoms of opioid withdrawal syndrome (wet-dog shakes, diarrhea, teeth chattering, ptosis, excessive grooming, and abnormal posture) were observed and recorded for 20 min after the injection (19). Body weight change 25 min after the naloxone injection also was measured as an additional index of the withdrawal syndrome. The stress procedure used for the two stress treatment groups was immobilization for 15 min/day in a distinct environment (a nearby room). Animals were immobilized by a finger-like immobilization apparatus (Centrap cage, Fisher Scientific, Inc.) every day prior to the solution self-administration period. Fluid consumption of water and the opioid solutions was determined by weighing the drinking bottles on an electronic balance. Amount of leaking and evaporation from the bottles was determined by weighing a drinking bottle after 7 h in the inverted position. The positions of the two bottles per cage were counterbalanced daily in the left and right positions to avoid the development of place preference or aversion.

At the end of the experiment, trunk blood was collected in heparinized tubes (50 μl, 10,000 IU/ml Na-heparin/tube) and centrifuged for 20 min at 1500 × *g* and 4°C. Brains were removed and stored at −70°C until assayed. Plasma levels of corticosterone were determined by radioimmunoassays (RIA) (Ventrex and ICN Biomedic). Plasma levels of morphine were determined by RIA (Diagnostic Products Corp., DPC). For the extraction of brain morphine, brains were homogenized in a 1 : 1 w/v solution of physiological saline. The homogenates were centrifuged at 35,5000 × *g* for 20 min at 4°C and aliquots of the supernatants were used in the RIA procedure (DPC). Plasma levels of fentanyl were analyzed by a modified fentanyl RIA kit for urine (DPC). The standards from the RIA kit were diluted by an equal volume of plasma from a drug-free animal to have the correct range of standards for plasma samples. For the extraction of brain fentanyl, brains were homogenized in a 1 : 2 w/v solution of 50% physiological saline and 50% absolute methanol. The homogenates were centrifuged at 35,500 × *g* for 20 min at 4°C. Aliquots of the supernatants were used in the RIA kit and standards were diluted by an equal volume of brain supernatant from a drug-free animal to have the correct range of standards for fentanyl extracted from brain.

Statistical Analyses

A 2 × 2 (stress condition × drug) repeated measures analysis of covariance (ANCOVA) was used for analyses of the consumption data. Proportions and amount of drug and water consumed during choice day 1 served as the covariates for the analyses of drug and water consumption during choice days, and baseline water consumption served as the covariate for the analysis of forced consumption days. Posthoc analyses used the Duncan multiple-range test. Data of the forced-consumption days were averaged across the 4 days within each cycle. Number of occurrences of withdrawal symptoms during the 20-min observation period were combined to form a total withdrawal score that was analyzed by a 2 × 2 analysis of variance (ANOVA). ANOVA also was used to analyze plasma and brain levels of morphine and fentanyl and plasma corticosterone levels. Significance level was determined at $\alpha = 0.05$.

RESULTS

Of the 50 animals, data from 48 were included in the analyses. Data from two animals were not included: One animal did not initiate morphine consumption yet lost substantial amounts of weight during the first week. A second animal consumed roughly five SD above the average of fentanyl solution during the forced-consumption days. Because there were no strain differences in the effect of stress on opioid consumption, data from the Sprague-Dawley and Wistar rats were collapsed within each treatment group.

Table 1 presents average plasma levels of corticosterone, plasma, and brain levels of morphine and fentanyl, dosage levels (mg/kg/day), fluid consumption data, and withdrawal measures. Plasma corticosterone levels were markedly higher in the two stress groups (S/M and S/F) compared to controls, $F(1,44) = 46.9$, $p < 0.05$, indicating that the stress manipulation was effective. During forced-consumption days, fentanyl groups consumed more of their solution than did the morphine groups, $F(1,43) = 7.29$, $p < 0.05$. However, there was no significant stress effect on opioid consumption during the forced consumption days, $F(1,43) = 1.12$, ns. For each drug, there were no significant differences between stress and control groups in plasma or brain opioid levels. There was

TABLE 1
AVERAGE PLASMA LEVELS OF CORTICOSTERONE, PLASMA, AND BRAIN LEVELS OF MORPHINE AND FENTANYL, DOSAGE LEVELS (mg/kg/day), OPIOID SOLUTION CONSUMPTION DURING FORCED-CONSUMPTION DAYS OF FIRST 25 DAYS, DRUG AND WATER CONSUMPTION DURING THE FIFTH CHOICE DAY, AND TOTAL WITHDRAWAL SCORE AND BODY WEIGHT CHANGE UPON NALOXONE CHALLENGE IN CONTROL AND STRESS GROUPS (±SE)

	Control/Morphine	Stress/Morphine	Control/Fentanyl	Stress/Fentanyl
Corticosterone (ng/ml)	90.5 ± 9.1	303.4 ± 77.2*	88.7 ± 10.6	349.4 ± 39.6*
Plasma morphine or fentanyl (ng/ml)	200.6 ± 33.2	192.8 ± 30.1	3.9 ± 0.7	5.0 ± 0.7
Brain morphine or fentanyl (ng/g)	30.5 ± 10.4	29.2 ± 5.4	3.6 ± 0.6	3.3 ± 0.3
Drug dosage (mg/kg/day)	46.3 ± 2.9	53.1 ± 3.7	1.6 ± 0.1	1.6 ± 0.1
Drug consumption during forced-consumption days (ml/day)	27.8 ± 2.4	31.4 ± 2.8	35.4 ± 2.3	35.1 ± 2.8
Drug consumption in the fifth choice day (ml/day)	11.5 ± 1.9	17.6 ± 2.6*	21.4 ± 2.9	34.2 ± 7.6*
Water consumption in the fifth choice day (ml/day)	19.6 ± 3.7	18.4 ± 2.3	17.1 ± 1.9	12.2 ± 2.3
Total withdrawal score †	16.1 ± 2.5	18.3 ± 3.4	5.1 ± 1.0	7.2 ± 2.6
Body weight change after naloxone injection (g)†	6.7 ± 1.1	6.1 ± 1.8	2.2 ± 0.4	1.8 ± 0.6

*Significant differences between groups within a drug class, $p < 0.05$.
†Significant main effect of drug, $p < 0.05$.

no significant stress effect on either of the two withdrawal measures. However, morphine groups showed more severe withdrawal syndrome upon naloxone challenge than did the fentanyl groups based on total withdrawal score and body weight change [$F(1,44) = 19.5$, $p < 0.05$, and $F(1,44) = 17.9$, $p < 0.05$, respectively].

The proportions of morphine and fentanyl intake during the five choice days are presented in Fig. 1. In contrast with the forced-consumption data, the choice data indicate that subjects exposed to stress consumed significantly more opioids than did subjects not exposed to stress [$F(1,43) = 5.5$, $p < 0.05$ and $F(1,43) = 6.6$, $p < 0.05$, for proportion and actual amount of drug consumed during choice days, respectively], fentanyl was consumed more than morphine [$F(1,43) = 6.7$, $p < 0.05$ and $F(1,43) = 6.1$, $p < 0.05$, respectively], and opioid consumption increased over time [$F(3,129) = 3.81$, $p < 0.05$ and $F(3,129) = 2.4$, $p < 0.05$, respectively]. Also, the stress × time interactions approached significance [$F(3,129) = 1.64$, $p < 0.1$ and $F(3,129) = 1.83$, $p < 0.08$, respectively], indicating a greater increase over time in drug

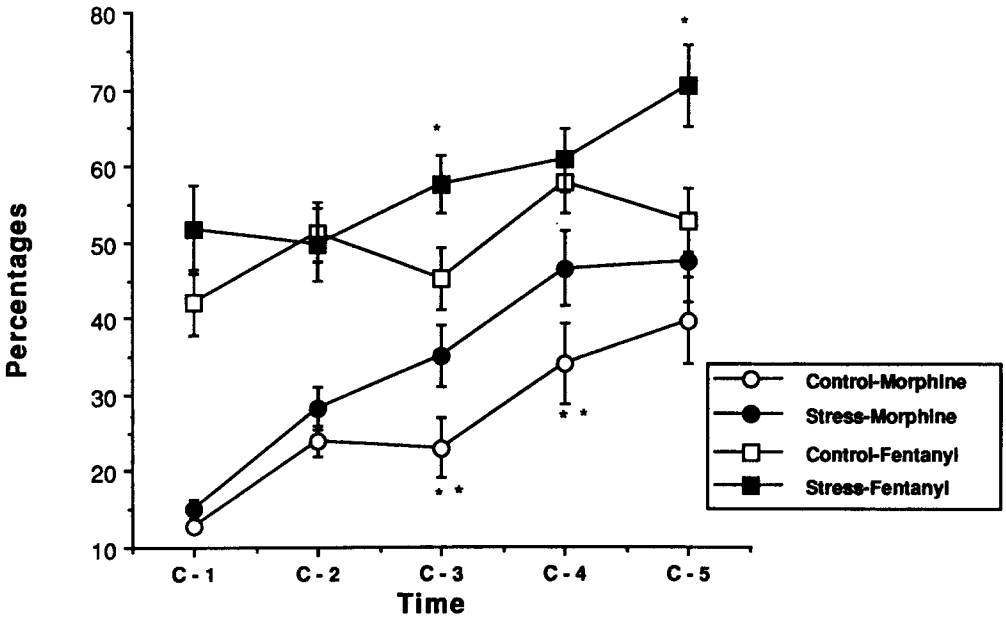

FIG. 1. Morphine and fentanyl consumption as percentages of total fluid consumption during choice days (±SE).

preference and consumption in stress groups compared to controls. No significant differences within each drug class were observed in water consumption during choice days.

In addition, stressed subjects with access to morphine ($n = 4$) that were examined over a longer time period clearly developed a preference for morphine on choice days 6–11 compared to their relevant controls ($n = 5$). Comparison of these two groups, using the dependent measure of proportion of morphine consumed during choice days, revealed a main effect of stress and a stress × time interaction [$F(1,7) = 5.4$, $p < 0.05$, and $F(5,35) = 3.85$, $p < 0.05$, respectively]. Morphine preference during choice days 10 and 11 was 68% and 70% of all fluid consumed in the stress group compared to 41% and 34% in controls, respectively. Figure 2 presents individual animals' morphine preference for these two subgroups during choice day 1 (after 4 days of access to morphine) and choice day 11 (55 days of access to morphine).

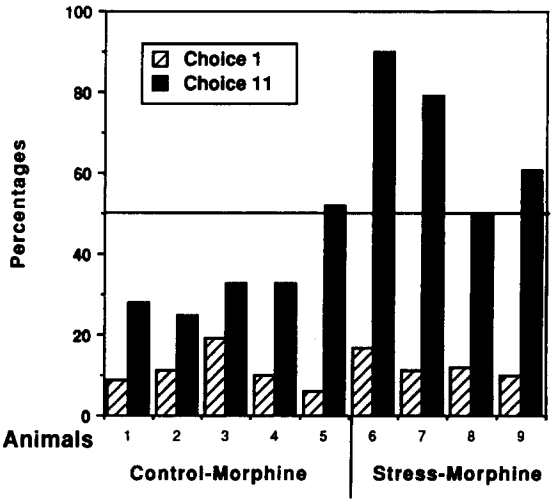

FIG. 2. Morphine consumption as percentages of total fluid consumption of individual animals on choice days 1 and 11.

DISCUSSION

The main hypothesis of this study was that stress would increase opioid self-administration in rats. The results obtained support this hypothesis. Rats exposed to 15 min/day of immobilization stress in a distinct environment increased their preference for morphine or fentanyl solutions compared to nonstressed controls. This effect was most dramatic in the animals that self-administered morphine for 2 months At the end of the study, these stressed animals showed a 70% consumption of morphine whereas the comparable control subjects showed a 34% morphine consumption. The oral morphine self-administration results for controls are similar to those previously reported [e.g., (14,27)] but higher than other reports [e.g., (1)]. In addition, the available morphine dosages (46–53 mg/kg) in this study are similar to those reported in previous studies of scheduled access of oral opioid consumption (1,8).

The withdrawal syndrome was more pronounced for morphine animals than for fentanyl animals. These results are somewhat surprising because fentanyl is thought to be about 80 times more potent than morphine as an analgesic (15), yet the dosage of fentanyl was about 1/30 of the morphine dosage. Perhaps the weak withdrawal syndrome observed in the fentanyl groups is related to the oral route of administration. It may be that first-pass liver metabolism is related to the moderate withdrawal syndrome observed in the fentanyl groups. In fact, the plasma levels of morphine and fentanyl (see Table 1) suggest that a higher rate of fentanyl first-pass metabolism occurred (i.e., plasma levels of morphine were about 40–50 times higher than those of fentanyl whereas morphine dosages were only about 30 times higher than fentanyl dosages).

Another finding of this study is that despite chronic opioid availability and repeated stress administration a marked increase in plasma corticosterone levels occurred in stress groups compared to controls. Previous studies using different types of stressors (e.g., laparotomy and histamine challenge) reported that chronic morphine administration inhibits corticosterone and ACTH responses to those stressors (4,21). It may be that the discrepant results are related to the route of drug administration. In the previous studies, morphine was injected rather than self-administered via the oral route. Interpretation remains speculative, but oral morphine administration elicits a reliable withdrawal syndrome upon exposure to naloxone challenge and does not inhibit plasma corticosterone release in response to immobilization stress.

In any study of the effect of stress (or any other environmental factor) on oral drug self-administration under limited fluid access, it is important to rule out two alternative hypotheses, namely, the effect of stress on taste and on thirst. The present data indicate that immobilization stress did not increase thirst. Specifically, no significant differences in fluid consumption were observed during the forced-consumption days during the first 25 days of the experiment. Fleetwood and Holtzman (8) further reported no differences in drug consumption between rats exposed to immobilization stress during a forced-consumption period of limited access to morphine solution (0.5 mg/ml) and a no-stress control group. To determine the effect of stress on sensitivity to a bitter taste, four Wistar rats were given a quinine solution (0.3 mg/ml dissolved in tapwater) according to the same procedure as the stress/morphine group for 5 choice days. Initial preference for this solution was 19% of total fluid consumption, similar to the 15% preference for the morphine solution during choice day 1. No systematic changes in preference for the quinine solution were observed during the other four choice days with a mean preference of 17%. Thus, it is unlikely that the effect of stress to increase oral opioid self-administration is related to a decreased sensitivity to the bitter morphine solution.

The mechanisms of the effect of stress on opioid use and abuse are not clear. The results of this study indicate that the effects of stress on opioid consumption and preference are not associated with decreases in brain or plasma morphine or fentanyl concentrations (see Table 1). Appelbaum and Holtzman (2) similarly found no differences in plasma and brain morphine levels in rats exposed to 30 min of immobilization stress compared to controls. At this point, we clearly have a paradigm to investigate the stress-opioid relationship, but we have no definitive data to support any particular explanation for this phenomenon.

We believe that the effect of stress on opioid self-administration may be related, in part, to conditioning factors associated with drug abuse [see (22,26,29)]. Specifically, principles derived from classical and operant conditioning paradigms may explain, in part, the positive relationship between stress and opioid use. This possibility merits empirical examination.

In summary, the present experiment used two different opioid drugs and found that immobilization stress increases consumption of those drugs. Based on this finding, future studies should examine possible mechanisms of the effect of stress on opioid self-administration under controlled experimental conditions. Elucidation of factors associated with the stress-drug interaction may lead to specific intervention techniques to help avoid initiation, increased opioid use, or relapse to opioid use.

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