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BRIEF COMMUNICATION

Potentiation of Morphine Analgesia in Rats Given a Single Exposure to Restraint Stress Immobilization

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CALCAGNETTI, D. J. AND S. G. HOLTZMAN. *Potentiation of morphine analgesia in rats given a single exposure to restraint stress immobilization.* PHARMACOL BIOCHEM BEHAV **41**(2) 449–453, 1992. – Rats exposed to restraint stress and injected with morphine show significantly greater increases in tail-flick latency compared to unstressed rats. However, it is not necessary for rats to be restrained at the time of testing to elicit a potentiated analgesic response to morphine. We reported recently that analgesia induced by 4.0 mg/kg morphine was significantly potentiated in rats that had been restrained for only 1 h at 24 h prior to testing. One purpose of the present study was to extend this observation by determining the ability of a single exposure to restraint stress to potentiate dose-dependently morphine (0.0, 2.0, 4.0, and 8.0 mg/kg)-induced analgesia in the tail-flick test. A second purpose was to assess the generality of the phenomenon by determining whether prior restraint would potentiate the analgesic effect of morphine in another common analgesic assay, the hot-plate test. Dose- and time-course (20–120 min) curves for morphine were generated in rats exposed to one of two treatments: no restraint stress (NS) and a single exposure to 1 h of restraint (RS). Rats subjected to 1 h of restraint and tested 24 h later displayed significant time- and dose-dependent potentiation (1.3–2.0-fold) of morphine-induced analgesia compared to unstressed rats in both the tail-flick and hot-plate tests. These results demonstrate that a single period of restraint is sufficient to activate the mechanisms responsible for potentiation of morphine-induced analgesia and that the degree to which stress modifies morphine's analgesia can be demonstrated using both the tail-flick and hot-plate assays.

Analgesia Morphine Restraint stress Tail-flick Hot-plate

THAT the magnitude and duration of opioid-induced analgesia is potentiated in rats exposed to restraint stress as compared to unstressed rats has been well documented in our laboratory (4–7,9,14). The mechanism by which restraint stress potentiates opioid analgesia is not known. All our previous studies used rats habituated (3–5 days) to restraint stress (of 1–4 h duration). We had hypothesized that habituation would maximize the changes produced by restraint and minimize variance during testing. In addition, analgesic testing was always performed while the rats were undergoing restraint. Recently, we demonstrated that a single 1-h exposure to restraint immobilization was sufficient to potentiate analgesia induced by 4.0 mg/kg morphine, even when the interval between restraint and analgesic testing varied from 24 h–1 week, in rats tested while unrestrained (10). One purpose of this study was to extend the observation that a single period of restraint is sufficient to potentiate the analgesic affect of morphine by generating complete morphine dose-response and time-course curves. Analgesic testing was conducted at 24 h after restraint because the degree of potentiation appears to peak at this interval between restraint and testing (10).

In previous studies from our laboratory, analgesia was measured by the radiant heat tail-flick assay. The results of early studies on the analgesic efficacy of opioid agonists concluded that the tail-flick response has the characteristics of a simple spinal reflex mechanism (13,16). It is known that forebrain systems also contribute to the mechanism(s) by which morphine produces analgesia (by using the hot-plate and formalin tests), requiring supraspinal as well as spinally organized responses (1,2,19). We hypothesized that restraint potentiation of opioid-induced analgesia also involves supraspinal as well as spinal brain mechanisms. Evidence of support of

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supraspinal sites mediating potentiation comes from the demonstration that intracerebroventricular injection of morphine (0.03–10 μg) (6), DAGO (0.01–0.3 μg), or DADLE (1.0–10 μg) (7) produce an equivalent degree of potentiation as does peripherally administered morphine (1.0–8.0 mg/kg) (14). In addition, it has recently been demonstrated that restraint-treated subjects given intrathecally administered DAGO (a μ selective opioid agonist) also results in significantly potentiated analgesia as revealed using both hot-plate and tail-flick assays (11). Given that both spinal and supraspinal mechanisms are involved in restraint-induced potentiation of opioid analgesia, we therefore employed the hot-plate assay (3) (Experiment 2) in the same design as we used for the tail-flick assay (Experiment 1) for comparative purposes, as well as to extend the generalizability of the results.

METHOD

Subjects

Naive, male rats of Sprague-Dawley descent were purchased from SASCO/King (Houston, TX). Subject body weights ranged from 275–375 g at the time of testing. Rats were housed three per cage and had food (Purina 5001) and tapwater ad lib. Testing took place in an isolated room maintained at about 22°C. A 12L:12D cycle was set with dark onset at 1900 h. Subjects were handled daily and testing began 6 days after arrival in our animal facility. All testing was conducted during the latter half of the light cycle, between 1600–1900 h.

Drug and Analgesic Testing

Morphine sulfate (Penick Corp., Newark, NJ) was dissolved in 0.9% saline (SA) on the day of testing. Saline served as the vehicle control injection. Doses are expressed as the free base. Morphine was injected SC in a volume of 1.0 ml/kg.

Analgesia was measured by the hot-plate assay with the surface temperature set at 55°C and by the radiant heat tail-flick assay (12), with modifications (15) as previously described in detail (8,10). The latency to tail-flick and rear paw-lick were recorded to the nearest second. A 6.0-s cutoff for the tail-flick assay and a 30-s cutoff for the hot-plate assay were established to minimize tissue damage. Each subject underwent three predrug trials that were conducted at 5-min intervals. The last trial served as the baseline measure for each subject. Tail-flick and paw-lick latencies were recorded 20, 40, 60, 90, and 120 min after drug injection for both experiments.

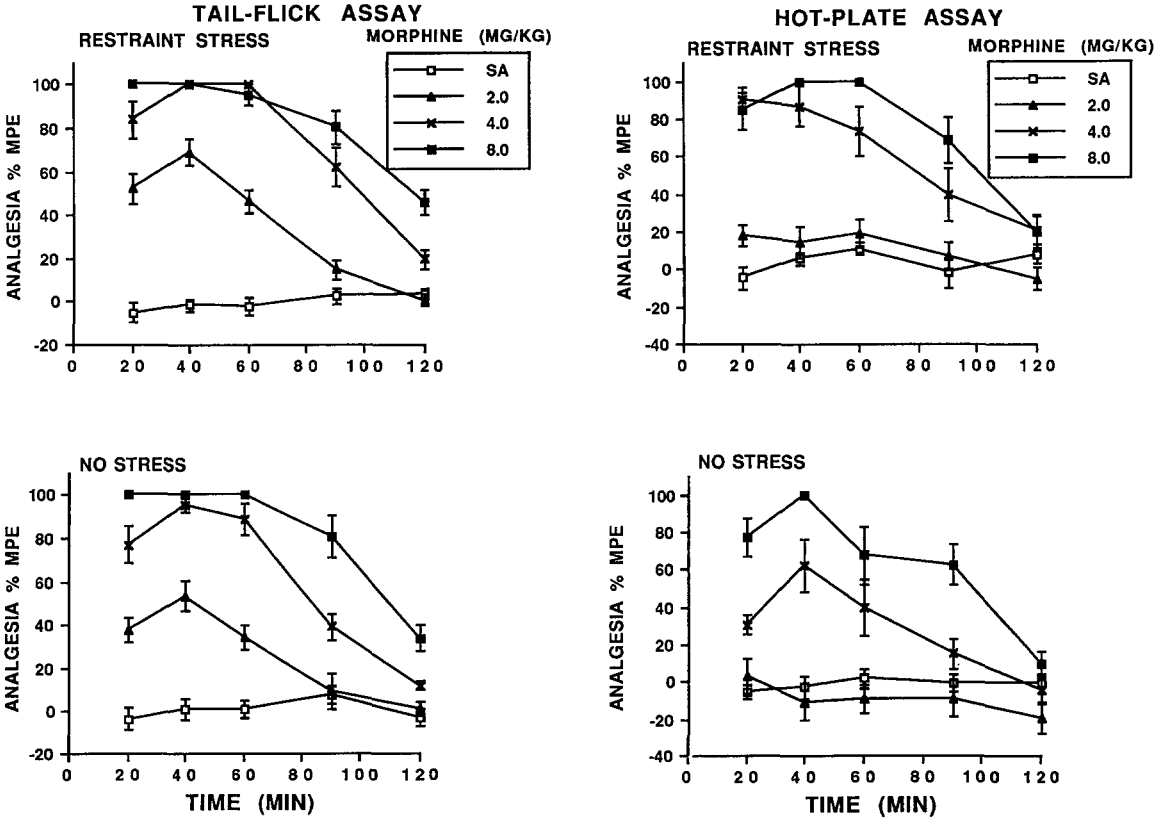

FIG. 1. Mean time course of tail-flick and paw-lick latency [expressed as % MPE (± SEM)] for rats that underwent restraint stress (top panels) and no stress (bottom panels) 24 h earlier. Rats were injected with one of four (SA, 2.0, 4.0, and 8.0 mg/kg) doses of morphine (n = 7–8 subjects per point). Tail-flick and paw-lick latencies for all experiments were sampled at 20, 40, 60, 90, and 120 min after injection. SA refers to saline, which served as vehicle control. Mean (± SEM) baseline tail-flick and paw-lick latencies (s) for rats that either underwent restraint stress (RS) or had not been subjected to 1 h of restraint stress (NS) 24 h prior to testing (n = 30–32 per group). Baseline tail-flick latency (s) and paw-lick latency in nonstressed rats was 2.30 (± 0.05) and 9.23 (± 0.55), respectively. Baseline tail-flick latency (s) and paw-lick latency in restraint-stressed rats was 2.46 (± 0.04) and 7.84 (± 0.47), respectively. The RS and NS baseline means significantly differed from each other ($p < 0.05$) using the tail-flick assay.

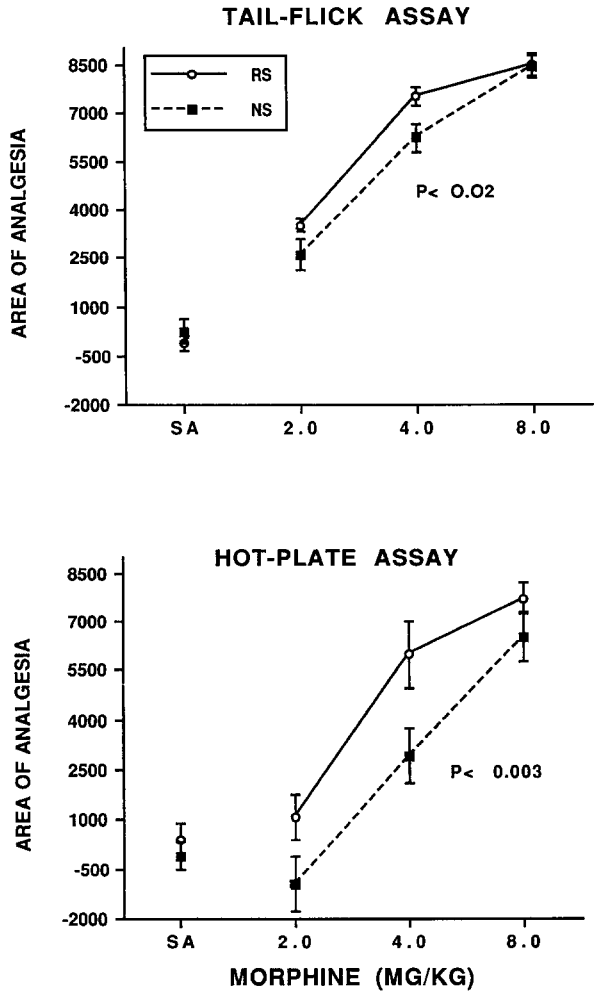

FIG. 2. Dose-effect curves for rats that either were (RS) or were not (NS) subjected to 1 h of restraint stress and then injected with one of four (SA, 1.0, 2.0, and 4 mg/kg) doses of morphine. Analgesia as indexed by the tail-flick (top panel) and hot-plate (bottom panel) assays is expressed as the area of analgesia derived from the area under the % MPE-time-course curves shown in Fig. 1. p values refer to the differences between the area under the respective RS and NA dose-response curves.

These experimental protocols were fully reviewed and approved by the Institutional Animal Care and Use Committee of Emory University.

Procedure

Subjects were randomly assigned to one of two treatment groups: 1) no stress (NS); 2) restraint stress (RS). Twenty-four hours prior to drug testing, the RS group was restrained beginning at 1600 h. Restraint was accomplished by immobilizing subjects for a single 1-h period in Plexiglas cylinders (5 to 6 cm in diameter), which were plugged with a #12 rubber stopper (a slice was removed so that the tail was freely mobile). Testing was initiated 24 h later with all rats unrestrained. Rats were weighed and underwent baseline testing. Within 5 min after baseline testing, subjects received SC injections of saline or morphine and either tail-flick latencies (Experiment 1) or paw-lick latencies (Experiment 2) were measured periodically beginning 20 min thereafter.

Data Treatment and Statistical Analysis

Both Experiments 1 and 2 conformed to a factorial design with two levels of restraint treatment (NS and RS) and four levels of morphine dose with repeated measures on the time of testing after morphine injection. Data from Experiments 1 and 2 were subjected to similar analyses. Tail-flick and paw-lick latency data are expressed as the mean percent maximum possible effect (% MPE) according to the following formula:

$$\% \text{ MPE} = \frac{\text{Postdrug Latency} - \text{Predrug Latency}}{\text{Cutoff time (s)} - \text{Predrug Latency}} \times 100$$

Dose-effect curves were derived by computing the area under the corresponding 20- to 120-min time-course-% MPE curves for each experiment. Areas were calculated using the trapezoidal rule (18). Statistical analyses were performed on the dose-effect curves by two-factor analysis of variance (ANOVA), with the level of statistical significance set at $p < 0.05$. The dose that produced 50% of the maximum analgesic effect (ED_{50}) was calculated by simple linear regression of the area of analgesia values for each subject and averaged for the group.

RESULTS

One-factor ANOVA of predrug baseline tail-flick latencies in Experiment 1 indicated that the NS and RS treatment groups reliably differed from each other, $F(1,60) = 8.2$, $p < 0.006$. Although the difference between RS and NS group means was 0.16 s, this difference was statistically reliable due to the large number of subjects (n = 30–31). However, one-factor ANOVA of baseline scores within each treatment group failed to reach statistical significance, $F_s < 1.7$, $p_s > 0.2$. It is not uncommon for RS-treated rats to show modest differences in baseline scores from those of animals in the corresponding control groups (4,14). One-factor ANOVA of Experiment 2 predrug baseline paw-lick latencies failed to reveal reliable differences between NS and RS treatment groups, $F(1,61) = 3.7$, $p > 0.006$. The mean and SEM baseline data for tail-flick and paw-lick latencies are presented in the legend of Fig. 1.

Figure 1 shows morphine time-dependent increases in tail-flick and paw-lick latencies for NS- and RS-treated rats. Two-factor ANOVA of postdrug tail-flick latencies transformed to area revealed significant main effects for treatment, $F(1,39) = 5.0$, $p < 0.015$, and dose, $F(2,39) = 122.5$, $p < 0.001$. Similar significant main effects were found for two-factor ANOVA of postdrug paw-lick latencies transformed to area; treatment, $F(1,40) = 10.9$, $p < 0.002$, and dose, $F(2,40) = 40.6$, $p < 0.001$. The interaction for both experiments failed to reach statistical significance. ED_{50} (95% confidence limit) values were calculated from the area of analgesia means (morphine doses 2.0–8.0 mg/kg) by simple regression. The ED_{50} in mg/kg for morphine-induced analgesia in rats that underwent restraint stress using the tail-flick and hot-plate assays were 2.62 (1.44–4.78) and 4.0 (1.54–10.60), respectively. The ED_{50} results of the tail-flick and hot-plate assays for rats that had not been subjected to 1 h of restraint stress 24 h prior to testing were 3.34 (1.86–6.00) and 6.0 (2.61–13.60), respectively. These results indicated that rats subjected to 1 h of restraint displayed potentiated morphine-induced analgesia compared to NS rats as demonstrated in both analgesic assays. For example, at the 4.0 mg/kg dose of morphine, RS-treated rats showed 1.3- and 2.0-fold more analgesia than NS-treated rats in the tail-flick and hot-plate assays, respectively.

The duration of morphine-induced analgesia was significantly increased in RS- as compared to NS-treated rats as

indexed using the tail-flick assay. For example, 90 min after 4.0 mg/kg morphine, RS-treated rats displayed greater than 60% MPE, whereas the analgesia of NS subjects had fallen below 40% MPE. Similar increases in RS-treated subjects were observed using hot-plate assay. However, the 2.0 mg/kg dose of morphine seemed to exert minimal analgesia in comparison to the large increases found with the tail-flick assay.

Dose-effect curves for NS- and RS-treated rats tested with the tail-flick and hot-plate tests are displayed in Fig. 2. The area under the corresponding % MPE-time course curves was calculated and used to construct dose-effect curves (expressed as the area of analgesia). The differences between RS and NS treatment group means (including ED_{50}'s and area of analgesia) are probably understated for two reasons: first, the necessity of using a cutoff, and, second, the latencies of several treatment groups failed to return to predrug levels within our 120-min observation period.

DISCUSSION

Our results demonstrate that rats injected with morphine (2.0–8.0 mg/kg) and tested 24 h after a single 1-h exposure to restraint display increased magnitude (1.3–2-fold) and duration of analgesia compared to NS-treated rats. These results confirm our previous findings in rats tested using the tail-flick assay and injected with a single dose of morphine (4.0 mg/kg) (10). As stated in the Results section, the baseline latency of the RS group was slightly but significantly higher (0.16 s) than that of the NS group in the tail-flick assay. This difference was unlikely to have contributed to the group differences in the effects of morphine. The baseline paw-lick latency of the RS group was *lower* (albeit not significantly so) than that of the NS group, yet the group differences in the effect of morphine were *larger* than those seen in the tail-flick assay.

Rats restrained for the first time display significantly greater magnitude (1.6-fold) and duration of analgesia than rats that had been habituated 5 days to restraint stress (10). We expand upon these findings by showing that rats exposed to a single session of restraint that undergo analgesic testing 24 h later with morphine show dose-dependent analgesia that is significantly increased in comparison to NS-treated rats. Our results also demonstrate that it is not necessary to habituate rats to restraint nor to test them within restraint tubes to show significant potentiation of morphine-induced analgesia.

In Experiment 2, we tested subjects using the hot-plate assay instead of the tail-flick. Our results demonstrate that in rats exposed to a single 1-h session of restraint stress potentiation of morphine-induced analgesia can be observed and quantified using the hot-plate assay. Based upon the ED_{50} values, the hot-plate assay is not as sensitive a measure of morphine analgesia as the tail-flick assay. For example, a 2.0-mg/kg dose of morphine produced minimal analgesia (scores did not exceed 20% MPE) on the hot-plate regardless of RS treatment, whereas robust analgesia was observed in the tail-flick assays (scores exceed 40% MPE). However, the hot-plate test was about equally sensitive in quantifying stress-induced potentiation.

We believe extending our observations with the tail-flick to the hot-plate assay supports the hypothesis that the mechanism(s) responsible for restraint-stress-induced potentiation of morphines' analgesic affect involve supraspinal brain sites. However, these results do not exclude a role for the contribution of spinally located mechanisms at which restraint stress may induce potentiation of morphine analgesia.

Lastly, some investigators have reported tolerance to morphine analgesia rather than a potentiation 3–7 days after stress (17). The factors that determine which of these two outcomes will occur remain to be elucidated. Nevertheless, it is clear that a relatively brief exposure to a stressful situation can result in long-lasting changes in the sensitivity to opioid-induced analgesia.

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