Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, No. 4, pp. 699-703, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00440-4

Rat Strain Differences in the Potentiation of Morphine-Induced Analgesia by Stress

DEDRA R. WOOLFOLK[1] AND STEPHEN G. HOLTZMAN

Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322

Received 21 June 1994

WOOLFOLK, D. R. AND S. G. HOLTZMAN. *Rat strain differences in the potentiation of morphine-induced analgesia by stress.* PHARMACOL BIOCHEM BEHAV **51**(4) 699-703, 1995.—Restraint stress has been shown to increase the magnitude and duration of morphine-induced analgesia; however, this phenomenon has only been investigated using the Sprague-Dawley rat strain. The purpose of this study was to determine if other rat strains would also exhibit a potentiated analgesic response to morphine compared to their unrestrained controls. Dose-response and time course curves for the analgesic effect of morphine (1.0, 3.0, 5.6, 10 mg/kg) were generated in adult, male Wistar, Lewis, Fischer 344, Long-Evans Hooded, and Sprague-Dawley rats either unrestrained or restrained in Plexiglas cylinders, using the tail flick assay. Morphine produced dose-dependent increases in tail flick latencies, and this effect was potentiated by restraint stress in the Sprague-Dawley, Wistar, Lewis, and Fischer 344 strains, but not in the Long-Evans Hooded rats. Because Sprague-Dawley and Wistar rats displayed the most robust stress effect, the use of either of these rat strains is appropriate in studying the mechanisms of stress-induced potentiation of analgesia. The differences among rat strains demonstrated in this study may serve as a basis for correlation with opioid function.

Rat strains Morphine Analgesia Restraint stress Opioids

NUMEROUS studies have shown differential responses of genetically inbred strains of rats to various physiological and behavioral manipulations. Lewis and Fischer 344 rats are known to differ genetically and have been used often to study possible genetic factors in various biological processes. For example, Fischer 344 rats self-administer opioids, cocaine, and ethanol to a lesser extent than do Lewis rats (11,17,20), whereas Lewis rats exhibit a greater conditioned place preference to morphine and to cocaine than do Fischer 344 rats (12). Also, Lewis and Fischer rat strains show differences in biochemical and electrophysiological parameters in the nucleus accumbens and the locus coeruleus that relate to their differential responses to opiate withdrawal; these results support the view that these strains may be useful in investigating the genetic factors that contribute to drug-related behaviors (13).

Outbred stains of rats can also differ from each other in the response to drugs such as morphine. For example, it has been shown that a low dose of morphine (5.0 mg/kg) produced hyperthermia and a high dose (40 mg/kg) produced hypothermia in Sprague-Dawley rats. Wistar rats, however, exhibited a hyperthermia in response to both doses of morphine (7).

It has been well documented in our laboratory that restraint stress potentiates the analgesic effect of morphine, as measured by the tail flick and hot plate tests (2,5,9); this potentiation is characterized by an increase in the magnitude and the duration of the analgesic effect. The mechanism by which restraint stress potentiates opioid analgesia is not known; however, there is evidence that stress induces the release of endogenous opioids (4,22), which appear to contribute to the potentiation of the analgesic effect of opioid drugs (1). This phenomenon has been studied primarily in Sprague-Dawley rats. Genetic factors contribute both to the response to opioids (11) and to the response to stress (6). The purpose of this study was to compare the effect of restraint stress on the analgesic effect of morphine in Fischer 344, Lewis, Wistar, and Long-Evans Hooded rat strains as well as in the Sprague-Dawley strain of rats.

METHOD

Subjects

Experimentally naive, male Sprague-Dawley, Fischer 344, Lewis, Wistar, and Long-Evans Hooded rats, all purchased from Charles River Laboratories (230-250 g at the time of

[1] To whom requests for reprints should be addressed.

purchase), served as the subjects. Rats were housed two to three per cage, maintained on a 12L : 12D cycle, and had unlimited access to food and water.

Analgesia

Analgesia was measured by the radiant heat tail flick assay (8), with modifications (10). The latency to tail flick was recorded to the nearest hundredth of a second, and a 6.0-s cutoff was established to minimize tissue damage. Each rat underwent two predrug trials conducted about 5 min apart. The mean of these trials served as the baseline measure for that subject. Tail flick latencies were recorded at 20-min intervals for 120 min after drug injection.

Procedure

All subjects were habituated for at least 3 consecutive days prior to drug testing by exposing them to the handling and testing procedures. Restrained and unrestrained animals of a particular strain were tested simultaneously on a given test day.

Subjects were randomly assigned to one of two groups: unrestrained (control) (Sprague-Dawley, Long-Evans Hooded, Fischer 344, Lewis, $n = 7$; Wistar, $n = 6$) or restraint stress (Sprague-Dawley, Long-Evans Hooded, Lewis, $n = 7$; Fischer 344, Wistar, $n = 8$). Rats in the control group were immobilized briefly by gently wrapping them in surgical towels for 15–20 s while tail flick latencies were measured. Five minutes after baseline testing, rats received SC injections of morphine (1.0, 3.0, 5.6, or 10 mg/kg). Animals in the restraint stress group were immobilized in Plexiglas cylinders plugged with rubber stoppers that had a slice removed such that the tail was freely mobile. Slats in the restraint device allowed for SC injections to be administered. Baseline latencies were determined 30 min after the onset of restraint, and rats received injections of morphine 5 min thereafter. The rats in the restrained group remained immobilized throughout the entire test session.

Drugs

Morphine sulfate (Penick Corporation, Newark, NJ) was dissolved in 0.9% saline and administered SC in a volume of 1.0 ml/kg. Doses are expressed as the free base.

TABLE 1

MEAN PREDRUG LATENCIES (95% CONFIDENCE LIMITS) OF RESTRAINED AND UNRESTRAINED RATS

Strain	Restrained (s)	Unrestrained (s)
Sprague-Dawley	4.03 (3.86–4.20)*	3.34 (3.13–3.54)
Wistar	2.76 (2.54–2.98)†‡	2.48 (2.31–2.66)§
Lewis	3.33 (3.18–3.47)†¶	2.51 (2.38–2.64)§
Fischer 344	3.37 (3.25–3.49)†‡¶	2.85 (2.62–3.08)
Long-Evans Hooded	3.05 (2.81–3.29)*‡	2.66 (2.44–2.88)§

*†Significantly different from corresponding unrestrained group, $*p < 0.01$, $†p < 0.05$.

‡Significantly different from restrained Sprague-Dawley, $p < 0.01$.

§Significantly different from unrestrained Sprague-Dawley, $p < 0.01$.

¶Significantly different from restrained Wistar, $p < 0.05$.

Statistical Analysis

Analgesia data are expressed as percent maximum possible effect (%MPE) according to the following formula:

$$\%\text{MPE} = 100 \times \frac{\text{postdrug latency} - \text{baseline latency}}{\text{cut-off time (6.0 s)} - \text{baseline latency}}$$

The mean baseline latencies shown in Table 1 represent an average of the baseline measures taken before all experiments. Differences between the baseline latencies of restrained and unrestrained rats of a particular strain were analyzed by Student's *t*-test. Dose-response curves were constructed from the

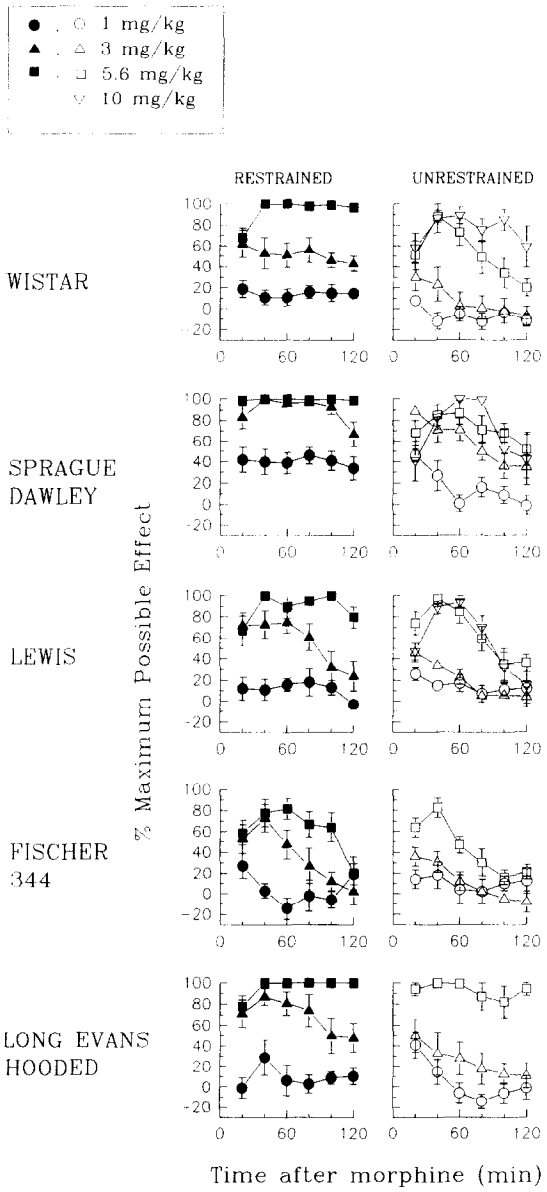

FIG. 1. Time course curves comparing the analgesic effect of 1.0, 3.0, 5.6, and 10 mg/kg morphine in Sprague-Dawley, Wistar, Lewis, Fischer 344, and Long-Evans Hooded rats. Drug doses were injected SC at time 0; tail flick latencies were measured at 20-min intervals after injection. Each point represents a mean ± SEM %MPE based on five to eight independent observations (see the Method section).

area under the corresponding 20- to 120-min time course-%MPE curves using the trapezoidal rule (21). The dose of morphine that produed an analgesic effect of 6000% MPE-min (ED_{50}) was calculated by linear regression. Statistical analyses were performed on the dose-response curves and the morphine ED_{50}s by two-factor analysis of variance (ANOVA), followed by Tukey's multiple comparison test. (The 10-mg/kg dose of morphine, tested only in the unrestrained groups, was not included in the ANOVA.) These tests were also used to evaluate differences in baseline latencies among strains. The level of statistical significance was $p < 0.05$.

RESULTS

All of the restrained rats of each strain displayed significantly higher baseline latencies than their unrestrained controls (Table 1). Two-factor ANOVA revealed a significant main effect for strain, $F(4, 92) = 30.3$, $p < 0.001$, and for restraint treatment, $F(1, 92) = 66.6$, $p < 0.001$. The interaction term was also significant, $F(4, 92) = 3.3$, $p = 0.014$. Restrained Sprague-Dawley rats had higher response latencies than restrained rats of any other strain, as did unrestrained Sprague-Dawley rats compared to unrestrained rats of the other strains tested. The stressed Wistar and Fischer 344 rats displayed significantly different baseline latencies from each other, and so did their unstressed controls. Although the baseline latencies of unrestrained Wistar and Lewis rats did not differ from each other, the baseline latencies of their restrained counterparts did.

Figure 1 displays the time course curves of 1.0, 3.0, and 5.6 mg/kg morphine in stressed rats and 1.0, 3.0, 5.6, and 10 mg/kg morphine in the unstressed rats. Morphine produced dose-dependent increases in tail flick latencies in restrained and unrestrained rats. Consistent with previous studies, the magnitude and duration of the analgesic effect were potentiated in most of the restrained groups. For example, at the 5.6-mg/kg dose of morphine, the stressed rats of the Sprague-Dawley and Wistar strains continued to exhibit 100% MPE 2 h after drug administration; in contrast, the analgesic responses of their unstressed controls began to decline approximately 1 h after drug injection. There were a few exceptions: the duration of the analgesic response to 5.6 mg/kg morphine did not differ between the restrained and unrestrained Fischer 344 rats; in addition, this dose of morphine produced an anal-

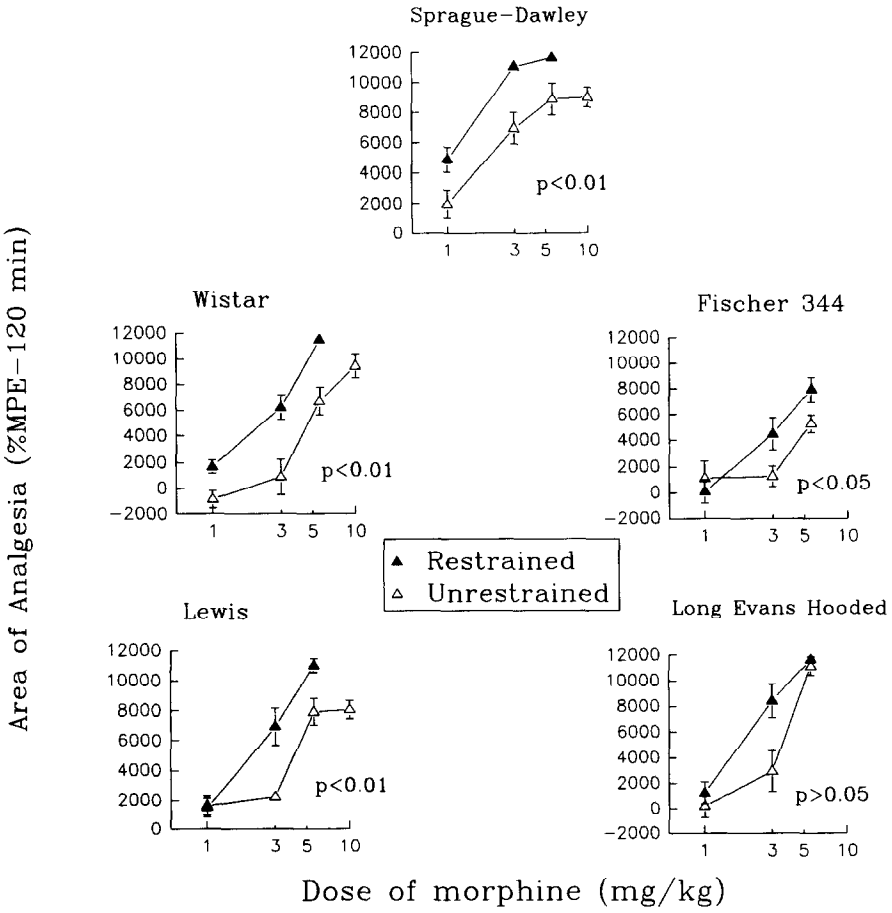

FIG. 2. Dose-response curves comparing the analgesic effect of morphine in restrained and unrestrained Sprague-Dawley, Wistar, Lewis, Fischer 344, and Long-Evans Hooded rats. Each point was derived from the area under the corresponding 120-min time course curves shown in Fig. 1 and represents a mean ± SEM based on five to eight independent observations (see the Method section). The p values refer to the differences between the area under the respective RS and NS dose-response curves.

gesic response of a similar magnitude and duration in both stressed and unstressed Long-Evans Hooded rats.

The area under the corresponding %MPE-time course curves was calculated and used to construct the dose-response curves in Fig. 2. Exposure to restraint stress resulted in leftward shifts of the morphine dose-response curves for each strain except the Long-Evans Hooded rat strain. Table 2 shows the calculated ED_{50}s and 95% confidence limits for morphine (the dose of morphine that produced an analgesic effect of 6000 %MPE-min) in each treatment group. Two-factor ANOVA revealed a significant main effect for strain, $F(4, 59) = 12.12$, $p < 0.001$, and for restraint treatment, $F(1, 59) = 43.22$ $p = 0.012$. The interaction term failed to reveal significance. There was approximately a 2-2.5-fold increase in the analgesic potency of morphine when paired with restraint stress compared to the unstressed control group.

DISCUSSION

Our results indicate that stress-induced potentiation of morphine-induced analgesia is not unique to Sprague-Dawley rats. Restrained Wistar, Lewis, and Fischer 344 rats also displayed increased analgesic responses to morphine compared to their unstressed controls. The potentiation of analgesia was characterized by increases in magnitude and duration of the effect, consistent with previous reports from our laboratory (2,9). This effect was largest in Sprague-Dawley and Wistar rats. Morphine was 2.6-fold more potent in restrained Sprague-Dawley rats than in the unrestrained rats. The analgesic effect of morphine in Wistar rats showed a 2.5 potency ratio. The potency of morphine was about 2.0-fold greater in restrained Lewis and Fischer 344 rats than in their unstressed controls. The potency of morphine was not significantly changed by restraint in Long-Evans Hooded rats. Similarly, the area under the morphine dose-response curves for the restrained and unrestrained Long-Evans Hooded rats did not differ. The restrained Long-Evans Hooded rats did show a significantly higher analgesic response to the 3.0-mg/kg dose compared to their unrestrained controls, but not to the 1.0- or 5.6-mg/kg dose.

Differences in morphine ED_{50}s across rat strains within the same treatment group could be due either to pharmacokinetic or to pharmacodynamic factors or a combination of both; it is not possible to determine which of these possibilities is correct based on the data from this study. Brain and plasma levels of morphine were shown to be comparable in stressed and unstressed Sprague-Dawley rats, suggesting that stress-induced potentiation of the analgesic effect of morphine is a phenomenon that is independent of drug dispositional factors (2). Therefore, it unlikely that pharmacokinetics is a factor in the potentiation of morphine-induced analgesia by restraint stress within the other rats strains tested, although this presumption remains to be established experimentally.

Sprague-Dawley rats displayed the highest baseline latencies under both the restrained and unrestrained conditions, as well as the largest effect of stress-induced potentiation of analgesia; however, there is no obvious relationship between the rank order of potency of morphine and predrug response latencies across rat strains. For example, the morphine ED_{50}s in unrestrained Sprague-Dawley and Long-Evans Hooded rats were not significantly different from each other, yet morphine was about 1.8 times more potent in the restrained Sprague-Dawley rats than in restrained Long-Evans Hooded rats. Restrained Fischer 344 rats had the highest ED_{50} of morphine (4.0 mg/kg) compared to the other groups of restrained rats (significantly different from Sprague-Dawley, see Table 2) and showed the smallest stress-induced potentiation of analgesia; however, both restrained and unrestrained Fischer 344 rats had the second highest predrug latencies. Restrained Long-Evans Hooded rats displayed higher predrug response latencies than their unrestrained controls, yet this strain did not exhibit a significant potentiation of the analgesic effect of morphine during restraint.

The data from this study differ in some respects from those in other rat strain comparisons in measures of responses to stress. For example, Rosecrans et al. (19) showed that Fischer 344 rats were significantly more sensitive to foot shock-induced analgesia than were Sprague-Dawley rats. Similarly, a study by He et al. (14) revealed a difference in the magnitude of lung injury induced by oxidant stress, where the lungs of Fischer 344, but not Sprague-Dawley, rats developed edema after short-term hypoxia. In our study, Sprague-Dawley rats were more sensitive to restraint stress and to stress-induced potentiation of morphine-induced analgesia than were Fischer 344 rats; however, the mean predrug latencies of Fischer 344 rats were lower, although not significantly so, than the mean latencies of Sprague-Dawley rats (Table 1).

In a study by Keim and Sigg (15), Fischer, Long Evans Hooded, Wistar, and Sprague-Dawley rat strains showed no significant differences in basal plasma corticosterone levels or in levels after restraint; however, corticosterone levels of Fischer 344 and Long-Evans Hooded rats recovered faster

TABLE 2

STRESS-INDUCED POTENTIATION OF MORPHINE ANALGESIA

Strain	ED_{50} (95% confidence limit, mg/kg)		Potency Ratio
	Restrained	Unrestrained	
Sprague-Dawley	1.1 (0.76-1.56)*	2.9 (1.92-4.51)	2.6
Wistar	2.3 (1.98-2.75)*	5.8 (3.25-10.42)	2.5
Lewis	2.3 (1.70-3.23)†	5.0 (3.53-7.18)	2.2
Fischer 344	4.0 (2.99-5.24)	7.6 (5.09-11.35)§¶	1.9
Long-Evans Hooded	2.2 (1.52-3.32)	3.1 (2.33-4.13)	1.4

*†Significantly different from restrained group ED_{50}, *$p < 0.01$, †$p < 0.05$.
‡Significantly different from restrained Sprague-Dawley, $p < 0.001$.
§Significantly different from unrestrained Long-Evans Hooded, $p < 0.01$.
¶Significantly different from unrestrained Sprague-Dawley, $p < 0.01$.

than did those of Sprague-Dawley and Wistar rat strains. This may be related to the finding in our study that Fischer 344 and Long-Evans Hooded rat strains displayed the smallest morphine potency ratios (1.9 and 1.4, respectively) out of the five strains tested.

Our study has shown that restraint stress potentiates morphine analgesia not only in Sprague-Dawley, but in Fischer 344, Lewis, and Wistar rats. This effect is not seen with Long-Evans Hooded rats. The differences among rat strains demonstrated in this study may, in fact, serve as a basis for correlations with opioid function. Unfortunately, there have not been many studies that have probed the differences in the endogenous opioid systems of the rat strains used in the present study; however, opioid receptor binding studies using various inbred mouse strains have shown differences in brain opioid receptor populations that correlate with sensitivity to opioids in various measures, such as analgesia, thermoregulation, and tolerance (3). This is likely to be the case for rats as well. Because Sprague-Dawley and Wistar rats displayed the greatest stress-induced increase in sensitivity to morphine, the use of either of these two rat strains would be appropriate in studying mechanisms of stress-induced potentiation of opioid-induced analgesia.

ACKNOWLEDGEMENTS

This study was supported, in part, by Grant DA00541 and Research Scientist Award KO5 DA00008, both from the National Institue on Drug Abuse, National Institutes of Health.

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