Pharmacology Biochemistry & Behavior, Vol. 20, pp. 631–633, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

BRIEF COMMUNICATION

Intermittent Cold Water Stress-Analgesia in Rats: Cross-Tolerance to Morphine

MARIE-NADIA GIRARDOT AND FRANK A. HOLLOWAY

Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center OMH Research Building, 306R, P. O. Box 26901, Oklahoma City, OK 73190

Received 28 February 1983

GIRARDOT, M.-N. AND F. A. HOLLOWAY. *Intermittent cold water stress-analgesia in rats: Cross-tolerance to morphine.* PHARMACOL BIOCHEM BEHAV 20(4)631–633, 1984.—Continuous cold water swim (CCWS, 3.5 min, 2°C) induces a non-opiate type of analgesia since 14 mg/kg of naltrexone or 20 mg/kg of naloxone only partially antagonize this stress-induced analgesia (SIA) and since there is no cross-tolerance between CCWS and morphine-analgesia. Intermittent cold water swim (ICWS) analgesia is significantly antagonized by naltrexone (14 mg/kg). These studies suggested that CCWS-analgesia is mediated by non-opioid systems, while ICWS-analgesia acts through a system that also mediates morphine analgesia. The hypothesis that ICWS-analgesia shares a common opioid pathway with morphine-analgesia, but not with CCWS-analgesia, was further tested by cross-tolerance studies in rats. The results showed a complete cross-tolerance to morphine analgesia in ICWS-tolerant animals, but no cross-tolerance to ICWS-analgesia in morphine-tolerant animals. This suggests that morphine- and ICWS-analgesia partially share a common pathway, ICWS acting probably at levels "downstream" from the opiate-sensitive sites, while CCWS induces analgesia by acting on a different system which is not mediated by opioids.

Stress Stress-tolerance Analgesia Morphine

ACUTE and severe stress induces a decreased responsiveness to nociceptive stimuli in rodents (for review, see [1, 2, 6, 10]). This stress-induced analgesia (SIA) generally adapts with chronic exposure. In some cases, cross-tolerance develops between SIA and morphine-induced analgesia [8, 9, 11, 12]. In other cases, there is no cross-tolerance between the two types of analgesia [5, 11, 12]. The various findings on cross-tolerance between specific stressors and morphine combined with their ability to be antagonized by opioid antagonists led to the conclusion that some stressors induce an opioid-mediated analgesia, while others produce analgesia by activation of non-opioid mechanisms.

Continuous cold water stress (CCWS, 3.5 min, 2°C)-induced analgesia was shown to adapt in rats exposed chronically (on 14 consecutive days) to the stressor [4], but there was no cross tolerance between this CCWS- and morphine-analgesia [5]. This finding combined with the demonstration that acute exposure to CCWS is only partially antagonized by high doses (20 mg/kg) of the opiate antagonist naloxone [3] led to the conclusion that CCWS-analgesia is not mediated by an opiate-sensitive system.

In a previous study [7], rats were exposed to intermittent cold water stress (ICWS); the resulting analgesia was significantly antagonized by naltrexone (14 mg/kg), while the same dose did not significantly antagonize CCWS-analgesia. These results suggested that endogenous opiates may be involved in intermittent, but not in continuous cold water swim-analgesia. The present study was undertaken to determine whether the analgesia induced by ICWS is cross-tolerant to morphine-induced analgesia. This would provide additional evidence for the involvement of the endogenous opiates in the analgesia induced by ICWS.

METHOD

Male albino Sprague-Dawley rats, weighing 300–350 g, were housed three to a cage, fed ad lib, and kept on a regular light-dark cycle (12:12). The experiments were performed during the light part of the cycle, between 0930 and 1200. Room temperature was kept constant (22–23°C).

Analgesia was quantified using a tail-flick test apparatus (Emdie Instruments) which measures the latency between the onset of a high intensity light beam focused on the tail and the occurrence of a spinally-mediated withdrawal reflex. Analgesia was measured by determining the mean of three tail-flick latencies at 40 second intervals. The light beam was applied sequentially at 5, 8, and 11 cm from the rostral end of the tail and automatically switched off after 15 seconds if no tail-flick occurred to avoid burning the skin.

The animals were submitted to either continuous or intermittent cold water stress. Some characteristics were common to both CCWS and ICWS. As such, the temperature of the water was 2°C. The depth of the water was at least 30 cm; the animals thus could not stand. The distance from

the surface of the water to the top of the container was approximately 70 cm; escape was therefore impossible. The parameters for the intermittent swim condition were: number of exposures: 18; frequency: 3/min; single duration: 10 sec; total time in water: 3 min. In the continuous CWS condition, the animals were swimming for 3.5 min.

The first experiment was related to the study of reciprocal cross-tolerance between ICWS- and morphine-analgesia. One group of 6 rats was sequentially injected with 5, 7.5 and 10 mg/kg of morphine sulfate (dissolved in 0.9% saline) at 5-day intervals. They were then submitted to the ICWS condition on 30 consecutive days. On days 16, 20, and 30 of this chronic ICWS regimen, they were not stressed but instead injected IP with 5, 7.5 and 10 mg/kg of morphine sulfate, respectively. The tail-flick latencies were measured on the days prior to the days of morphine injection to ensure that these three interruptions in the chronic ICWS treatment did not alter tolerance. Another group of 6 rats was injected on 15 consecutive days with 10 mg/kg of morphine. On Day 16, they were submitted to the ICWS condition. In these two groups of rats, the tail-flick latencies were measured prior to and 30 minutes after either morphine injection or ICWS.

In the second experiment, the effect of 10 mg/kg of morphine in rats submitted to either chronic CCWS, ICWS, or morphine treatments was studied. Four groups of rats (n=6 in each group) were used. One group served as controls, and was injected with 10 mg/kg of morphine. The second, third, and fourth groups were submitted to, respectively, 10 mg/kg of morphine injection, ICWS or CCWS every day on 15 consecutive days. On day 16, all animals in these 3 groups were injected with 10 mg/kg of morphine. The tail-flick latencies were measured prior to, and 30 and 60 minutes after morphine administration.

RESULTS

The results of the first experiment are illustrated in Fig. 1. The mean baseline tail-flick latencies in the various conditions ranged from 5 to 6 sec. In this figure, the level of analgesia is represented as an analgesia index which is derived from the tail-flick latencies (TFL) according to the equation:

$$\text{A.I.} = \frac{\text{TFL 30 min after treatment} - \text{baseline TFL}}{\text{15 (cut-off point)} - \text{baseline TFL}}$$

The A. I. in the animals submitted to chronic morphine or ICWS conditions were significantly lower than the A. I. in the acute condition ($p<0.01$, t-test), implying that tolerance to the analgesic effect of both morphine and ICWS did develop. Morphine's effect was significantly lower after than before ICWS treatment ($p<0.05$, 0.01, and 0.01 for 5, 7.5 and 10 mg/kg of morphine, respectively, using a t-test), while there was no significant difference between the A.I. in the morphine-tolerant rats subsequently submitted to ICWS and the A.I. following an acute exposure to ICWS in non-treated animals. These results suggest that there is cross-tolerance to morphine in rats tolerant to ICWS, but that this cross-tolerance is unidirectional; rats tolerant to chronic treatment of morphine are not tolerant to ICWS.

In the second experiment, tolerance developed in the chronic morphine and ICWS conditions. The increases from baseline of the tail-flick latencies at 30 min after treatment (Δ=TFL at 30 min − baseline TFL) were compared on days 15 and 1 for the various conditions. On day 15, the mean Δ represented 22 and 27% of the mean Δ on day 1 in the morphine and ICWS conditions, respectively. They were significantly different for these 2 conditions ($p<0.02$ for the morphine-treated animals, $p<0.001$ for the group submitted to ICWS). Interestingly, in the rats submitted to the chronic CCWS, the mean Δ significantly decreased during the first 7 days of treatment (mean Δ on Day 7 vs. Day 1, significant at $p<0.001$), but they then progressively increased from Day 8 to Day 15, at which they no longer differed significantly from the Δ on Day 1 ($0.05<p<0.1$). In this CCWS condition, the mean Δ on day 15 represented 77% of the mean Δ on day 1.

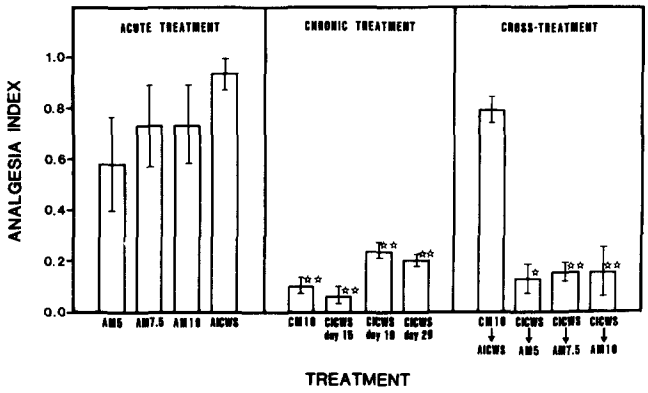

FIG. 1. Analgesia Index (mean±S.E.M.) as a function of either acute, chronic, or cross-treatment between morphine and intermittent cold water stress. Abbreviations: AM5-acute morphine (5 mg/kg); AM7.5-acute morphine (7.5 mg/kg); AM10-acute morphine (10 mg/kg); AICWS-acute intermittent cold water swim; CM10-chronic morphine (10 mg/kg); CICWS-chronic intermittent cold water swim. There were 6 animals in each group. Statistical significance using the t-test method and comparing the chronic treatment groups to the acute groups subjected to identical drug or stress treatment, and comparing the cross-treatment groups to the acutely treated groups subjected to identical drug or stress treatment. *and **—The difference was significant at $p<0.05$ and $p<0.01$, respectively.

Figure 2 illustrates the effect of 10 mg/kg of morphine in 4 different groups of animals (controls, morphine-tolerant, ICWS-tolerant, and CCWS-tolerant). The results were analyzed using the ANOVA method. While the mean baseline latencies were not significantly different in the 4 groups, $F(3,44)<1$, the overall tail-flick latencies differed significantly, $F(3,20)=7.78$, $p<0.005$; significant differences were obtained at both 30 minutes, $F(3,44)=10.31$, $p<0.001$, and 60 minutes, $F(3,44)=8.17$, $p<0.001$, following injection. There was no significant overall difference between the morphine- and ICWS-tolerant animals; neither were they different at 30 and 60 minutes after injection. This was also the case for the controls versus CCWS-treated animals. The morphine-tolerant animals and the CCWS-treated animals were significantly different at all time levels from the controls and ICWS-tolerant animals, respectively ($p<0.01$).

DISCUSSION

Bodnar *et al.* [4] reported complete tolerance development with exposure to a 3.5 min CCWS on 14 consecutive days, but a lack of cross-tolerance to morphine in the CCWS-tolerant animals. Our finding of a diphasic analgesic

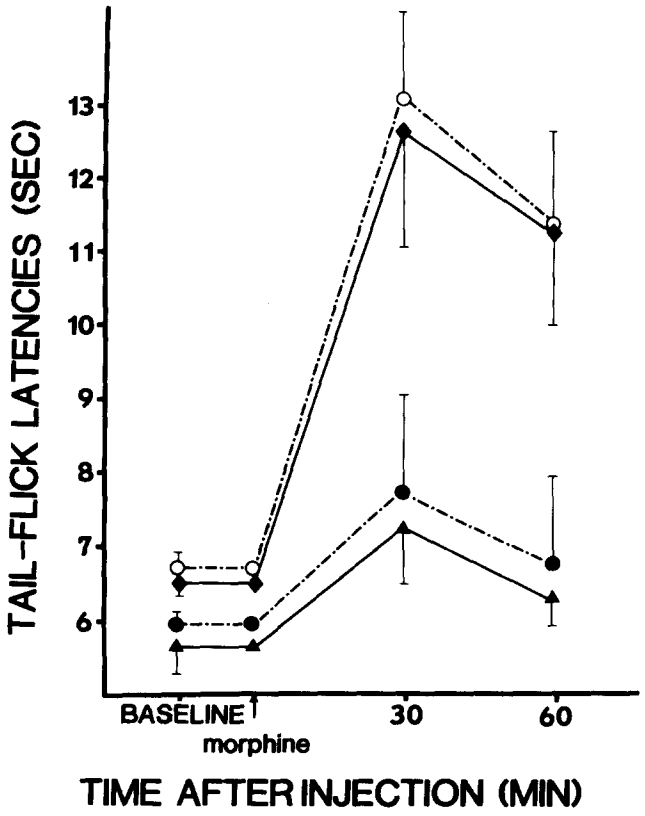

FIG. 2. Tail-flick latencies prior to, right after, 30 and 60 min after injection of 10 mg/kg of morphine (at arrow) in 4 groups of rats (n=6 in each group). One group served as controls (full line, diamonds). The other 3 groups were submitted to either CCWS (interrupted line, open circles), ICWS (interrupted line, closed circles), or injection of 10 mg/kg of morphine (full line, triangles) on 15 consecutive days. The morphine injection was done on the day following the chronic treatments.

response to chronic CCWS with progressive loss of tolerance from day 8 to day 15 of treatment deviates somewhat from this earlier report. The discrepancy may be due to differences in the procedure used to test for analgesia. Indeed, in the previous report, analgesia was measured using the flinch-jump threshold method, while we used the tail-flick test. The present results, however, confirm the previous finding that analgesia induced by CCWS is not cross-tolerant to morphine.

In the animals submitted to the intermittent CWS condition (and although the animals spent approximately the same amount of time in the water as in the CCWS condition) cross-tolerance to morphine did develop. As previously suggested, it thus seems that the pattern of the stressor is one determinant of the type of analgesia-regulating system involved. We have shown [7] that 14 mg/kg of naltrexone does not significantly antagonize the analgesia induced by CCWS, while it significantly antagonizes the analgesia induced by ICWS. This finding, which suggests that the opioid analgesic system is involved in the second but not the first condition, combined with the present demonstration of cross-tolerance to morphine in ICWS-tolerant animals and no cross-tolerance to morphine in CCWS-treated animals strongly suggests that the pattern of the stressor is, at least in the case of cold water stress, an important factor resulting in the involvement of an opioid-mediated analgesic system.

The cross-tolerance between ICWS- and morphine-analgesia is only unidirectional; indeed, morphine-tolerant rats are not tolerant to ICWS. That reciprocal cross-tolerance does not always exist between analgesia-inducing stressors and/or morphine has already been reported [11]. Interpretation of this lack of reciprocal cross-tolerance between morphine and intermittent CWS can only be speculative at this point. One possibility is that acute ICWS may induce analgesia by acting at levels "downstream" from an opioid sensitive site on a pathway inhibiting the spinal pain neurons.

ACKNOWLEDGEMENTS

This research was supported by NIDA training grant 5 T32 DA07105-05 and a minigrant from the Research Council, Department of Psychiatry and Behavioral Sciences. The authors wish to thank Lynn Montgomery for her skilled secretarial assistance.

REFERENCES

1. Amir, S., Z. W. Brown and Z. Amit. The role of endorphins in stress: Evidence and speculations. *Neurosci Biobehav Rev* **4:** 44–86, 1980.
2. Bodnar, R. J., D. D. Kelly, M. Brutus and M. Glusman. Stress-induced analgesia: Neural and hormonal determinants. *Neurosci Biobehav Rev* **4:** 87–100, 1980.
3. Bodnar, R. J., D. D. Kelly, A. Spiaggia, C. Ehrenberg and M. Glusman. Dose-dependent reductions by naloxone of analgesia induced by cold water stress. *Pharmacol Biochem Behav* **8:** 667–672, 1978.
4. Bodnar, R. J., D. D. Kelly, A. Spiaggia and M. Glusman. Stress-induced analgesia: Adaptation following chronic cold water swims. *Bull Psychonomic Soc* **11:** 337–340, 1978.
5. Bodnar, R. J., D. D. Kelly, S. S. Steiner and M. Glusman. Stress-produced analgesia and morphine-produced analgesia: Lack of cross-tolerance. *Pharmacol Biochem Behav* **8:** 661–666, 1978.
6. Chance, W. T. Autoanalgesia: Opiate and non-opiate mechanisms. *Neurosci Biobehav Rev* **4:** 55–67, 1980.
7. Girardot, M. N. and F. A. Holloway. Cold water stress-analgesia in rats: Differential effects of naltrexone. *Physiol Behav,* 1984, (in press).
8. Lewis, J. W., J. E. Sherman and J. C. Liebeskind. Opioid and non-opioid stress analgesia: Assessment of tolerance and cross-tolerance with morphine. *J Neurosci* **1:** 358–363, 1981.
9. Miczek, K. A., M. L. Thompson and L. Shuster. Opioid-like analgesia in defeated mice. *Science* **215:** 1520–1522, 1982.
10. Millan, M. J. Stress and endogenous opioid peptides: A review. *Mod Probl Pharmacopsychiatry* **17:** 49–67, 1981.
11. Spiaggia, A., R. J. Bodnar, D. D. Kelly and M. Glusman. Opiate and nonopiate mechanisms of stress-induced analgesia: Cross-tolerance between stressors. *Pharmacol Biochem Behav* **10:** 761–765, 1979.
12. Watkins, L. R., D. A. Cobelli, P. Faris, M. D. Aceto and D. J. Mayer. Opiate vs. non-opiate footshock-induced analgesia (FSIA): The body region shocked is a critical factor. *Brain Res* **242:** 299–308, 1982.