Pharmacology Biochemistry & Behavior, Vol. 31, pp. 933–935. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

BRIEF COMMUNICATION

Effect of Morphine on Self-Stimulation in Rats and its Modification by Chloramphenicol

ROBERT L. COPELAND, JR. AND S. N. PRADHAN[1]

Department of Pharmacology, Howard University College of Medicine, Washington, DC 20059

Received 28 March 1988

COPELAND, R. L., JR. AND S. N. PRADHAN. *Effect of morphine on self-stimulation in rats and its modification by chloramphenicol.* PHARMACOL BIOCHEM BEHAV 31(4) 933–935, 1988.—The effect of morphine was studied on self-stimulation (SS) behavior in rats implanted with bipolar electrodes in the posterior hypothalamus. A single dose (10 mg/kg) of morphine decreased SS responding within 10–20 min, reaching a minimum level between 20–40 min after which the responding gradually returned to normal. The SS responding then increased above the control level at 120–180 min postdrug, then slowly returned to normal, thus showing a rebound effect. The combination treatment with morphine (10 mg/kg) and chloramphenicol (50 mg/kg) on SS behavior produced an accentuation of the initial decrease in responding, which was prolonged before gradually returning to the control levels without showing any rebound effect. The data suggest that alterations in protein synthesis may underlie the suppressed excitatory effect of a high dose of morphine on SS behavior.

Morphine Chloramphenicol Self-stimulation Posterior hypothalamus

ACUTE administration of morphine has been reported to produce biphasic effects on the responding for intracranial self-stimulation (SS), that have been described as an initial decrease in lever-pressing with a gradual return to normal, then followed by a late stimulatory phase of activity before returning to normal (1,12). In addition, it was also observed that with daily injections of morphine, tolerance developed to the inhibitory effect, while its excitatory effect had an earlier onset and tended to be enhanced (8). This late stimulatory effect may be described as a 'rebound' effect, that may be further enhanced after chronic administration leading to development of physical dependence and reflected as a component of the abstinence syndrome.

There is an indirect evidence which suggests that tolerance to and dependence on morphine may involve activity and/or synthesis of certain macromolecules capable of counteracting the drug effects. Several investigators have demonstrated that development of tolerance to the analgesic effect of morphine could be attenuated by inhibitors of protein and/or nucleic acid synthesis, such as actinomycin D, 6-mercaptopurine, 5-fluorouracil, cycloheximide, puromycin and chloramphenicol (5, 6, 10, 11). However, many of these drugs produce serious toxic effects. Therefore, in the present study effects of morphine were studied over a prolonged time-course demonstrating the rebound effect and attempts were made to inhibit the rebound effect by a protein synthesis inhibitor, such as chloramphenicol which is comparatively less toxic and is clinically used.

METHOD

Animals

Male F344 rats with initial body weights of 250–350 g obtained from Charles River Breeding Laboratory were used. These rats were housed individually in a light- and humidity-controlled room, fed with rodent laboratory chow and allowed free access to drinking water.

Experimental Procedures

Rats were anesthetized with sodium pentobarbital (40 mg/kg, IP) and bipolar stainless-steel electrodes (Plastic Products) were stereotaxically implanted in the posterior hypothalamus (PH), according to the procedure of Nimitkipaison *et al.* (14). Verification of the location of the electrode tips was histologically done in 2 of the rats upon completion of the experiments. After implantation and recovery from surgery, rats were trained for the SS behavior as in our previous experiment (14), to press a lever once to obtain

[1]Requests for reprints should be addressed to Dr. S. N. Pradhan.

reinforcing electrical stimulation. The electrical stimulus was a biphasic symmetical square wave, consisting of 0.1-msec pulse width, delivered at 100 Hz for a 0.3-sec train duration. They were subjected to daily 4 1/3-hr sessions for 6 days a week. The animals were placed in the test chamber and allowed to press for the reinforcing stimulation at the same time each day.

Treatment with drugs was initiated as soon as the performance of each rat reached a stable rate (e.g., lever-pressing rate during a session remained within 10% of the daily average for 5 to 6 days) usually after training for 2 to 3 weeks. Recordings of the lever-pressing were made at 20-min intervals. The day prior to a test of a specific drug, the animals were injected with saline or sterile water (i.e., the vehicle for the drugs). There was at least 7 days gap between drug treatments.

Drugs and Administration

Morphine sulfate (Mallinckrodt; dose calculated as the base) was dissolved in saline. Chloramphenicol (LyphoMed, Inc.; dose calculated as the base) was used as the monosuccinate sodium salt and dissolved in sterile water. Both drugs were freshly prepared and given IP in doses indicated in the Results section. Morphine was injected following a 20-min preinjection control period at the beginning of a session. Chloramphenicol was given 60 min prior to injection of saline or morphine administration, then the animals were returned to the Skinner box.

Analysis of Data

For calculation and evaluation, differences between response rates (lever-pressing/20-min period) for the preinjection control and the treatment (with saline or drug) periods were determined for each animal on both the saline-treatment day (control) and drug-treatment days. Means of these differences from corresponding periods on the saline-treatment day and the drug-treatment day were compared by analysis of variance (ANOVA) using a general linear model procedure to assess the overall effects of drugs and time periods. Specific within-time period comparisons were performed by ANOVA with the Bonferroni correction (13) for multiple comparisons, controlling the type I error rate at $p<0.05$.

RESULTS

The effects of morphine on SS behavior was studied in five rats which were treated with a single dose of morphine (10 mg/kg, IP), chloramphenicol (50 mg/kg, IP) and the combination of morphine and chloramphenicol. During the control sessions, SS responding of rats used in these experiments had a mean value of 769±39/20-min period.

Immediately after the preinjection (control) recording the animals were injected with saline or morphine and returned to the Skinner box. Morphine (10 mg/kg) caused an initial decrease in SS respondings with the maximum effect at 40 min, and persisted for 60–80 min. The responding gradually returned to normal by 100 min, then increased above the control level at 120–180 min and later slowly returned to normal by 220 min, thus showing a rebound effect (Fig. 1). Statistical analysis by ANOVA for the overall comparison between control (saline-treated) and morphine-treated rats showed a significant ($p<0.04$) difference. Specifically, within-time period comparisons by ANOVA with the Bonferroni correction showed a significant ($p<0.04$) decreased at

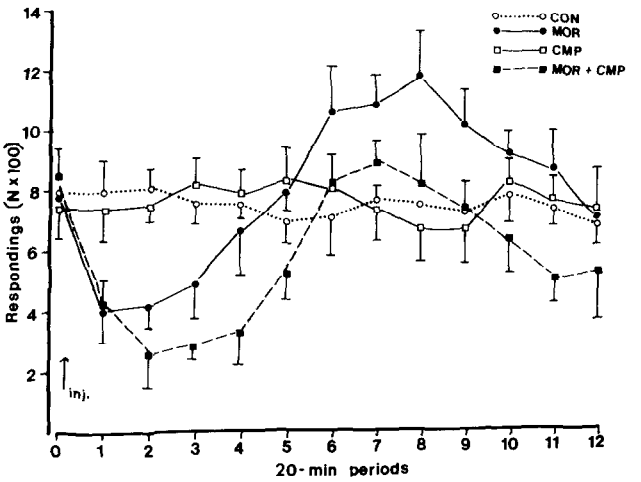

FIG. 1. Time-courses of effects of a single dose of morphine (10 mg/kg, MOR), chloramphenicol alone (50 mg/kg, CMP) or their combination on self-stimulation during 4 1/3-hr sessions in 5 rats. Saline was injected during the control (CON) session. Dots with line denote the mean ± S.E.; inj., injection.

40 min, and significant increases ($p<0.04$ and $p<0.03$) at 120 and 140 min respectively.

Administration of chloramphenicol alone caused little or no change in responding during the 4 1/3 hr session (Fig. 1). In rats pretreated with chloramphenicol 60 min prior to administration of morphine, studying the effects of combination treatment showed that the initial decrease in lever-pressing was accentuated up to 80 min compared to the saline control, before the responding slowly returned to the normal level. There was a slight increase in responding above the control level at 140 min, but statistically insignificant, thereby showing that the rebound effect was markedly attenuated (Fig. 1). The within-time period comparisons by ANOVA with the Bonferroni correction showed a significant ($p<0.005$ to 0.03) decrease from 20–80 min. Further, comparison between morphine and morphine-chloramphenicol groups showed a significant ($p<0.05$) difference at 120–160 min postdrug.

DISCUSSION

The present study demonstrates the biphasic effects of morphine on the SS behavior with an initial depressive phase, followed by a delayed stimulation at 120–140 min postdrug. While this study is basically in agreement with those of Lorens and Mitchell (12) and Bush *et al.* (3), other investigators (9, 15, 17) have shown only its depressant effect on SS responding. The discrepancy in findings between these studies may be attributable to differences in the site of the brain stimulation, baseline response rate, schedule of treatment, duration of the observation period and other factors. In a separate study from our laboratory (4), the biphasic effects of morphine on SS behavior have been correlated with similar biphasic changes in the neurochemical profile following administration of morphine corroborating the rebound phenomenon.

The present study further shows that the combination treatment of morphine and chloramphenicol accentuates the initial depressant phase of morphine and suppresses the subsequent stimulatory or rebound effect. Accentuation of the

depressant effect appears to be due to suppression of the stimulatory effect that might have partially masked and encroached on the later part of the depressant effect which is now unmasked. In other neurochemical studies from this laboratory it was shown that the combination treatment of chloramphenicol with morphine attenuated the rebound increase in dopamine levels in the caudate nucleus and the diencephalon at 160 min postdrug. Such effect of chloramphenicol was also demonstrated through its inhibition of morphine tolerance in rats at doses which produce in brain tissues a drug concentration corresponding to that shown to be effective in vitro to markedly reduce ^{14}C-leucine incorporation into synaptosomal proteins (7). Ambrose and Coons (2) reported that chloramphenicol is capable of inhibiting protein synthesis in maturing or actively proliferating mammalian cells, as well as newly-induced antibody synthesis. In these cases, chloramphenicol would act by interfering with a fast turnover of mRNA induced in the cell under a special stimulus situation. Although the brain cells of an experimental animal are already mature and differentiated, it has been suggested (16) that some functional aspects of the nervous system concerning information processing and adaptation responses to the dynamic state of the environment might resemble the induction of antibodies in immunocytes, including appearance of special types of RNA at the synaptic level which may be sensitive to chloramphenicol.

The adaptive process(es) which occur(s) during the development of tolerance and dependence following chronic administration of morphine can be reflected as rebound effects in the SS behavior. The rebound effect may represent an overcompensation of narcotic actions during the abstinence syndrome in an attempt to maintain homeostasis. Moreover, the manifestations of the central and autonomic stimulation shown during narcotic withdrawal are reflected in the enhancement of SS responding during the late stimulatory phase even after a single dose of morphine.

REFERENCES

1. Adams, W. J.; Lorens, S. A.; Mitchell, C. L. Morphine enhances lateral hypothalamic self-stimulation. Proc. Soc. Exp. Biol. 140:770–771; 1972.
2. Ambrose, C. T.; Coons, A. H. Studies on antibody production. VIII. The inhibitory effect of chloramphenicol on the synthesis of antibody in tissue culture. J. Exp. Med. 117:1075–1088; 1963.
3. Bush, H. D.; Bush, M. F.; Miller, M. A.; Reid, L. D. Addictive agents and intracranial stimulation: Daily morphine and lateral hypothalamic self-stimulation. Physiol. Psychol. 4:79–85; 1976.
4. Copeland, R. L., Jr.; Pradhan, S. N. Effect of morphine on central neurotransmitters in the rat. Biogenic Amines 5:205–214; 1988.
5. Cox, B. M.; Osman, O. H. Inhibition of the development of tolerance to morphine in rats by drugs which inhibit ribonucleic acid or protein synthesis. Br. J. Pharmacol. 38:157–170; 1970.
6. Della Bella, D.; Frigeni, V. Inhibition of protein synthesis and tolerance to opiates. In: Adler, M. L.; Manara, L.; Samanin, R., eds. Factors affecting the action of narcotics. New York: Raven Press; 1978:403–412.
7. Della Bella, D.; Frigeni, V.; Sassi, A. Control of tolerance reversal by protein synthesis inhibitors. In: Kosterlitz, H. W., ed. Opiates and endogenous opioid peptides. Amsterdam: Elsevier/North Holland; 1976:353–360.
8. Glick, S. D.; Rapaport, G. Tolerance to the facilitatory effect of morphine on self-stimulation of the medial forebrain bundle in rats. Res. Common. Chem. Pathol. Pharmacol. 9:647–652; 1974.
9. Holtzman, S. G. Comparison of the effects of morphine, pentazocine, cyclazocine and amphetamine on intracranial self-stimulation in the rat. Psychopharmacologia 46:223–227; 1976.
10. Kaneto, H.; Kosaka, N.; Hirota, N. Timing of cycloheximide administration and inhibition of the development of analgesic tolerance to morphine. Life Sci. 30:2351–2354; 1982.
11. Loh, H. H.; Shen, F-H.; Way, E. L. Inhibition of morphine tolerance and physical dependence development and brain serotonin synthesis by cycloheximide. Biochem. Pharmacol. 18:2711–2721; 1969.
12. Lorens, S. A.; Mitchell, C. L. Influence of morphine on lateral hypothalamic self-stimulation in the rat. Psychopharmacologia 32:271–277; 1973.
13. Miller, R. J., Jr. Simultaneous statistical inference. New York: Springer Verlag; 1981:519.
14. Nimitkipaisan, Y.; Bose, S.; Kumar, R.; Pradhan, S. N. Effects of L-dopa on self-stimulation and brain biogenic amines in rats. Neuropharmacology 16:657–661; 1977.
15. Olds, J.; Travis, R. P. Effects of chlorpromazine, meprobamate, pentobarbital, and morphine on self-stimulation. J. Pharmacol. Exp. Ther. 128:397–401; 1960.
16. Ramirez, G. Synaptic plasma membrane protein synthesis: Selective inhibition by chloramphenicol *in vivo*. Biochem. Biophys. Res. Commun. 50:452–458; 1973.
17. Schaefer, G. J.; Holtzman, S. G. Dose- and time-dependent effects of narcotic analgesics on intracranial self-stimulation in the rat. Psychopharmacology (Berlin) 53:227–234; 1977.