Psychopharmacology 65, 65–68 (1979)

Psychopharmacology

Facilitation of Opiate- and Enkephalin-Induced Motor Activity in the Mouse by Phenytoin Sodium and Carbamazepine

R. J. Katz* and Kristine Schmaltz

Mental Health Research Institute, Department of Psychiatry, University of Michigan Medical Center, Ann Arbor, MI 48109, U.S.A.

Abstract. In the first experiment, adult male Swiss-Webster mice were systemically injected with a standard dose of morphine. Compared to the influence of vehicle, the motor activity of morphine-injected mice was increased. Neither phenytoin sodium nor carbamazepine alone facilitated motor activity, but pretreatment with both drugs further facilitated the increased motor activity produced by morphine. In a second experiment, mice were injected centrally with a long-acting analog of leu-enkephalin. It also increased motor activity in comparison with vehicle. Again, both phenytoin sodium and carbamazepine further facilitated this response. Both experiments suggest a facilitatory interaction between some aspects of these anticonvulsants and opiate-induced motor activity.

Key words: Anticonvulsant drugs – Carbamazepine – Enkephalin – Morphine – Motor – Opiate – Phenytoin sodium – Psychomotor activity

Morphine, enkephalin, and electrical stimulation of endorphin-rich brain areas are known to produce both local changes in multiple unit activity and more general electrocortical effects, including seizures. (Frenk et al., 1978; Rhodes and Liebeskind, 1978; Tortella et al., 1978; Urca et al., 1977). These findings are consistent with and extend prior studies establishing a stereospecific and naloxone-reversible proconvulsant effect of a number of opiates (e.g., Mannino and Wolf, 1974, 1975) and may have additional functional significance. Local changes in unit firing rate, especially within the periaqueductal grey area (PAG), correlate with analgesia (Rhodes and Liebeskind, 1978; Urca et al., 1977), while EEG seizures in other brain areas (e.g., cortical seizures) have been correlated with catalepsy and signs of opiate withdrawal such as wet dog shakes and twitches (Frenk et al., 1978). It is therefore possible that local PAG unit changes and more general electrocortical alterations in fact mediate different aspects of the morphine response. Given the above possiblity, we tested interactions of opiates and an enkephalin analog with drugs which have clinical efficacy in reducing seizures as one of their actions. The models we used for investigating these effects involved motor activity in grouped and individual mice after systemic morphine or central D-Ala^{2}Leu Enkephalinamide, respectively. This model draws upon the initial studies of Goldstein and Sheehan (1969), and related effects have been found by us (Carroll and Sharp, 1972; Katz et al., 1978a, b) and others (e.g., Bhargava, 1978; Pert and Sivit, 1977).

In the present two experiments, we tested animals with and without phenytoin sodium and carbamazepine, which are known to be clinically effective in reducing seizures (Woodbury and Fingl, (1975). Experiment I examined interactions with morphine, and Experiment II utilized running, with an enkephalin analog as the basis of comparison. Our findings suggest these compounds may selectively facilitate opiate-induced activity.

Experiment I

Experiment I examined the effect of drug pretreatment upon morphine-induced running in grouped mice.

General Materials and Methods

Subjects. Experimentally naive, adult male Swiss-Webster mice ($N = 245$) (30–40 g) (Charles River Farms, Portage, MI) maintained on ad libitum food (Teklad 4.0% fat diet S-0836) and tap water, with automatically programmed 12-h day-night cycles (lights on 8 a.m.). Group housing was employed for the first experiment with four to six subjects per cage. Individual housing was employed in the second experiment to protect surgically implanted cannulae.

Apparatus. In both experiments field sensitive activity monitors (Stoelting, Chicago) were used, calibrated to within 5% of each other.

* To whom offprint requests should be sent

0033-3158/79/0065/0065/$01.00

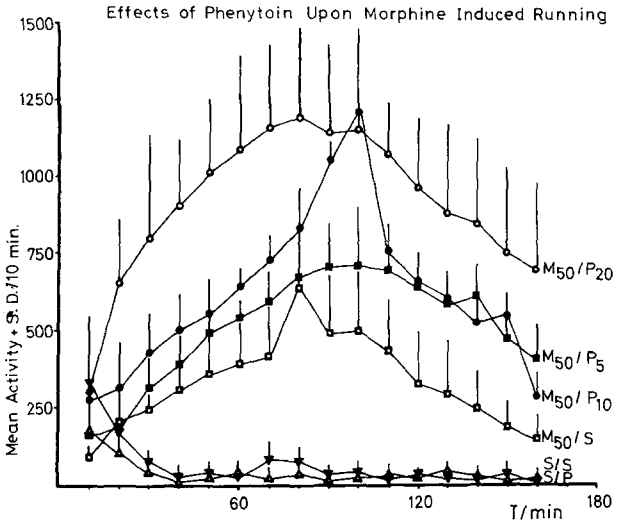

Fig. 1. Phenytoin facilitation of morphine-induced running in the mouse. Each data point represents the mean ± SD of the activity counts for each experimental group at indicated times: Saline vehicle (S); △ phenytoin (P) (20 mg/kg); □ morphine (M) (50 mg/kg); ■ Morphine (50 mg/kg) + phenytoin (5 mg/kg); ● morphine (50 mg/kg) + phenytoin (10 mg/kg); ○ morphine (50 mg/kg) + phenytoin (20 mg/kg)

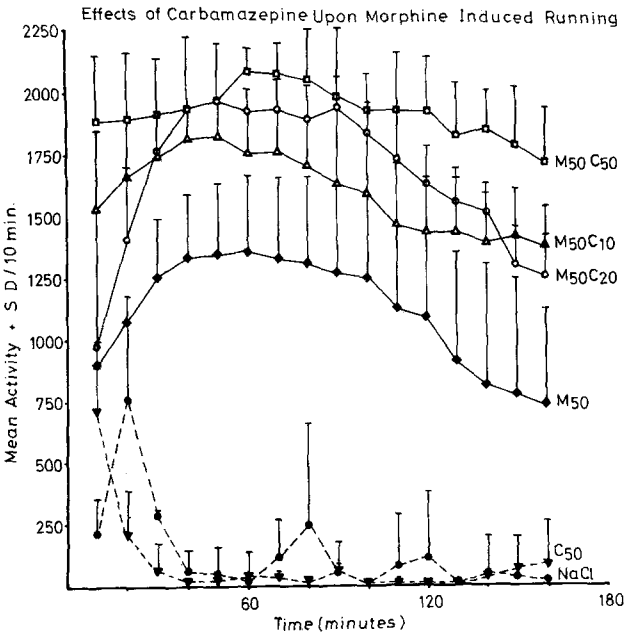

Fig. 2. Carbamazepine facilitation of running induced in mice by morphine. Mean ± SD. Symbols: ● Saline vehicle (S); ▲ carbamazepine (C); ◆ morphine (M) (50 mg/kg); △ morphine (50 mg/kg) + carbamazepine (10 mg); ○ morphine (50 mg/kg) + carbamazepine (20 mg/kg); □ morphine (50 mg/kg) + carbamazepine (50 mg/kg)

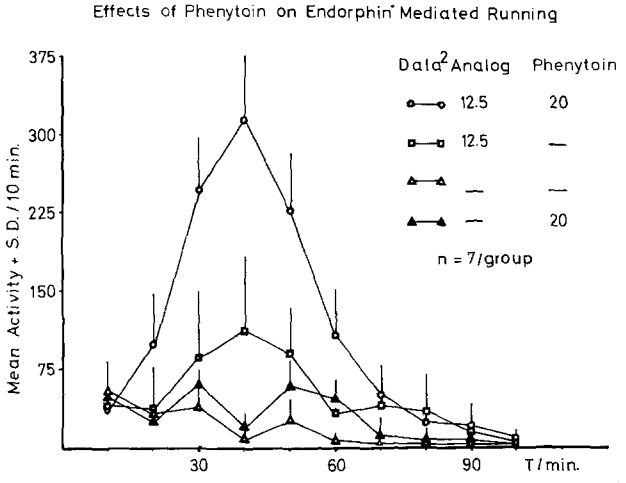

Fig. 3. Effects of phenytoin sodium upon the running responses of individual mice to an enkephalin analog. Mean ± SD. Drugs administered 10 min and immediately prior to recording

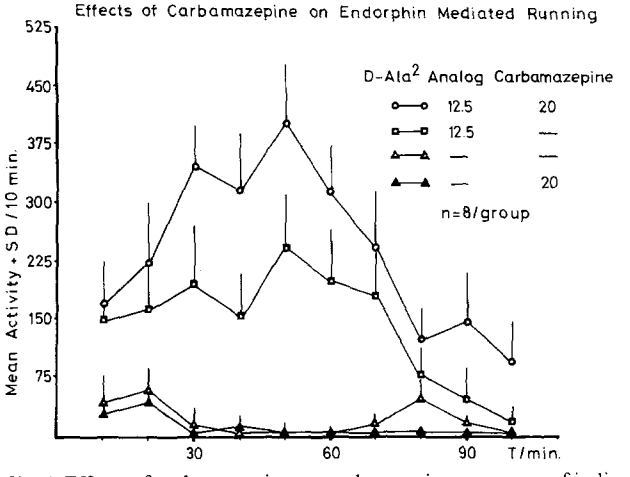

Fig. 4. Effects of carbamazepine upon the running responses of individual mice to an enkephalin analog. Mean ± SD. Drugs administered 10 min and immediately prior to recording

Testing was carried out in an illuminated environment with a continual masking noise of 25 dB. The activity monitors were set at the intermediate setting of the apparatus with a sensitivity of 30. The apparatus was recalibrated once during the experiment as noted below. Each testing cage (51 × 41 × 22 cm polypropylene cages, Scientific Products series 70) was placed upon the monitor with a fresh bedding of pine chips. Since composition of the bedding may affect drug metabolism (Vesell et al., 1973) we note that similar results regarding activity may be obtained on many different forms of bedding, or without any bedding whatsoever. Moreover, all such metabolic effects are at least to our knowledge chronic in nature as opposed to acute exposure. Additional details of apparatus and procedure have been published previously (Katz et al., 1978a, b).

It will be noted that all groups in Figs. 2 and 4 are higher than in Figs. 1 and 3. This includes both control and experimental animals. This was due to a recalibration of sensors between the two experiments, which yielded higher readings across conditions for a standard amount of movement. We emphasize these relative changes do not reflect altered running in the two figures, but merely measurement differences over several months.

Drugs and Injection Procedure. Morphine sulphate (Wyeth) was given at a dose of 50 mg/kg IP. Phenytoin sodium (Dilantin, Parke-Davis) was similarly administered in doses of 5, 10, and 20 mg/kg. Carbamazepine (Tegretol, Ciba-Geigy) was injected at 10, 20, and 50 mg/kg. A 0.9% saline vehicle was used for all injections including

controls, except for carbamazepine which was injected as suspension in 1% Tween-80 solution in saline again with the appropriate vehicle serving as control. All injections were administered 1 ml, 0.1 kg and were based on the established pharmacology of each compound (Woodbury and Fingl, 1975).

Behavioral Procedure. Morphine-injected mice were tested with cage mates in groups of four. Cage mates exceeding four were routinely discarded to standardize testing. This represented a similar proportion (20%) for all groups. Habituation began at 9 a.m., with initial injections 1–2 p.m. The initial injection of phenytoin, carbamazepine, or vehicle was followed 5 min later by an injection of morphine or vehicle. Experimental recording began immediately after the second injection. Ten minute recording intervals were used throughout the next 180 min. A minimum of seven determinations (i.e., at least seven observations of the activity of grouped subjects) was made for each data point (range 7–11) for phenytoin, and three to eight determinations were made for carbamazepine.

All data are presented as means and SE and were analyzed by analyses of variance.

Results

During initial exposure mice displayed initially high activity (3000–4000 counts/10 min) which declined monotonically over a 2–3-h period. The final 2 h were characterized by low (less than 50 counts/10 min) or no activity. All subjects showed zero activity immediately preceding injection. This allowed a sensitive baseline for evaluating drug facilitation. Morphine produced high stable rates of activity in comparison to the vehicle (Figs. 1 and 2, respectively). Increased activity was not seen after either phenytoin or carbamazepine given in the absence of morphine. However, phenytoin augmented the behavioral response to morphine in a dose-related manner (Fig. 1) and carbamazepine had a similar effect (Fig. 2).

To insure approximate homogeneity of variance, scores were transformed prior to analysis (square root of counts plus 1). Analysis was then by treatments-x-subjects analysis of variance with correction for repeated measures (Bruning and Kintz, 1977). Effects evaluated were dose of drug, time course of drug action, and interaction of these factors.

Analyses of variance indicated significant effects of groups [$F(5,48) = 16.7$], time [$F(15,720) = 9.3$], and interaction [$F(75,720) = 4.3$] ($P < 0.001$ in all cases) in the initial phenytoin experiment. For the carbamazepine experiment, groups [$F(5,18) = 13.3$], time [$F(15,185) = 9.1$], and interaction [$F(75, 185) = 3.7$] were again significant ($P < 0.001$ in all cases).

Experiment II

In Experiment I the antiepileptic drugs phenytoin and carbamazepine facilitated the normal running response of grouped mice to morphine. Experiment II was intended to extend the generality of this finding in three ways. A novel opiate (D-Ala^2 substituted leu-enkephalinamide) was used to induce running. Mice were individually tested, and the peptide was injected centrally. Central injections were deemed especially important since it might be argued, from past experiments, that the behavioral facilitation represented an effect upon drug disposition rather than on the central activity of opiates. Direct injection into the brain minimized the possibility of major changes in the former.

Materials and Methods

Surgical and Behavioral Procedure. Except as indicated below apparatus and procedures were identical to Experiment I. Mice were anesthetized with 80 mg/kg IP sodium pentobarbital (Nembutal), and stereotaxically implanted with a permanently indwelling 23 gauge stainless steel cannula constructed from a hypodermic syringe. The cannula was aimed at the lateral ventricle using coordinates from Slotnick and Leonard (1975) and secured to the skull with stainless steel screws and acrylic dental cement. A removable wire obturator was used to maintain patency and normal cleanliness. Seven days were allowed for recovery from surgery. Histology at the close of testing infolved injection of 5 µl of commercially available black ink; animals were killed 5–10 min later and the ventricular surface was examined. Only subjects showing diffusion of ink throughout the ventricles were included in the analysis.

The behavioral procedure consisted of five initial habituation sessions of 4 h each. Subjects were individually exposed to the testing chambers without any injections. After habituation, subjects were exposed to the chambers between 4–5 p.m. and allowed to habituate to a point of no movement as registered upon the sensors. This generally required an additional 3 h. Each mouse was then removed and briefly injected with either vehicle or drug in four combinations (vehicle alone, phenytoin or carbamazepine alone, enkephalin analog alone, combined treatment with phenytoin or carbamazepine, and enkephalin analog). A minimum of 48 h separated the test periods. Order of injection was systematically rotated across subjects with all subjects receiving all treatments. Activity was recorded for ten blocks of 10 min each.

Drugs. Based upon previous experiments in our laboratory (Katz et al., 1978a, b) and Experiment I, the following drug doses were employed: 12.5 µg of D-Ala^2 leu-enkephalinamide (Peninsula 8619; Tyr D-Ala Gly Phe Leu-NH); 20 mg/kg of both phenytoin (Warner Lambert/Parke Davis) and carbamazepine (Ciba-Geigy). For the analog and its vehicle 5 µl of Ringer-Locke solution was microinjected within 30 s through the permanently indwelling cannula by use of a manually operated Hamilton microsyringe. The recording session began immediately after injection. The test drugs were administered IP (1 mg/0.1 kg) using a 0.9% sodium chloride vehicle solution for phenytoin and a 1% Tween-80 in saline suspension vehicle for carbamazepine. The latter drugs were administered 10 min prior to the start of the session.

It should be noted that the dose of enkephalin analog was a relatively modest one for the production of running (Katz et al., 1978a) and the doses of phenytoin and carbamazepine represented relatively high and moderate doses respectively (see Experiment I).

General Results

Details of the statistical analysis are presented in Experiment I. However, the present analysis utilized a complete factorial design as described by Bruning and Kintz (1977). Again a transformation based upon the

square root +1 was used to initially equate variance. The results of Experiment II are consistent with the first experiment in that a low dose of opiate produced a modest running response that was increased up to threefold by the other drugs. Figure 3 presents the results for phenytoin effects upon D-Ala^2 leu-enkephalinamide which are significant for treatments, time, and interaction (respectively, $F = 8.6$, $df = 3,18$ $P < 0.01$; $F = 14.1$, $df = 9,54$, $P < 0.001$; $F = 4.3$, $df = 27,162$, $P < 0.001$). Figure 4 presents similar results for carbamazepine, again main effects and interaction are significant (respectively, for groups, time, and interaction, $F = 5.1$, 8.7, 3.7; $df = 3,21$, 9,56, 189, $P < 0.05$, 0.001, 0.001).

Discussion

Based upon the present findings of potentiation of an opiate-induced behavioral syndrome, it appears possible that some aspects of motor behavior which are affected by phenytoin or carbamazepine normally inhibit murine activity after opioids. The pharmacological and biochemical differences of two anticonvulsants are considerable (Woodbury and Fingl, 1975). This further supports the possibility that their shared antiepileptic activity contributes to the findings, since structurally dissimilar compounds have the same effect. While one mechanism for the action of these drugs rests with their effects upon electrocortical seizures, it would be premature to state unequivocably that this is the sole mediator of the observed effect. Alterations in sensory activity and other pharmacological actions cannot be disregarded as potential contributors. Clearly an examination of EEG patterns would be helpful in further elucidating these effects.

In Experiment II the same anticonvulsant drugs potentiated the murine activity response to a potent and long-lasting analog of an endogenous CNS peptide. This is clearly consistent with Experiment I and, given the procedural modifications with respect to the latter, argues that the observed effect is not dependent upon drug disposition. In addition to demonstrating the predicted drug interaction, these results confirm earlier reports from our laboratory demonstrating a running response to enkephalin analogs (Katz et al., 1978a).

Earlier findings suggested that at least some forms of morphine-induced activation were seizure-dependent, for example, phenytoin pretreatment was reported to eliminate opiate-induced feline mania (Fertziger et al., 1974). Phenytoin also has been shown to reduce morphine-induced catalepsy in rats (Cookson and Mann, 1978). It may be concluded that the responses induced by morphine must be evaluated carefully within and across species and testing situations.

Acknowledgements. The technical assistance of Giulio Baldrighi and editorial assistance of Ester Washington are gratefully acknowledged. We are grateful to Ciba Geigy and Warner Lambert Parke Davis for their generous donations of drugs.

References

Bhargava, H. U.: Effects of methionine enkephalin and morphine on spontaneous locomotor activity, antagonism by naloxone. Pharmacol. Biochem. Behav. **9**, 167–171 (1978)

Bruning, J. L., Kintz, B.: Computational hanbook of statistics. Glenview: Scott Foresman 1977

Carroll, B. J., Sharp, P. T.: Monoamine mediation of the morphine activation in mice. Br. J. Pharmacol. **46**, 124–139 (1972)

Cookson, S. L., Mann, J. D.: Reversal and prevention of acute morphine induced catalepsy in naive rats (abstract). Neuroscience **4**, 488 (1978)

Fertziger, A. P., Stein, E. A., Lynd, J. J.: Suppression of morphine-induced mania in cats. Psychopharmacologia. **36**, 185–187 (1974)

Frenk, H., McCarty, B. C., Leibeskind, J. C.: Different brain areas mediate the analgesic and epileptic properties of enkephalin. Science **200**, 335–337 (1978)

Goldstein, A., Sheehan, P.: Tolerance to opioid narcotics. Tolerance to the running fit caused by levorphanol in the mouse. J. Pharmacol. Exp. Ther. **169**, 175–184 (1969)

Katz, R. J., Carroll, B. J., Baldrighi, G.: Behavioral activation by enkephalins in mice. Pharmacol. Biochem. Behav. **8**, 493–496 (1978a)

Katz, R. J., Carroll, B. J., Leibler, L.: Enhancement of drug induced motor activity by an inhibitor of phenylethanolamine-N-methyl-transferase. Neurosci. Letters **8**, 83–88 (1978b)

Mannino, R. A., Wolf, H. H.: Opiate receptor phenomenon: Proconvulsant action of morphine in the mouse. Life Sci. **15**, 2089–2096 (1974)

Mannino, R. A., Wolf, H. H.: Morphines proconvulsant action: Importance of endogenous norepinephrine. Life Sci. **16**, 1659–1668 (1975)

Pert, A., Sivit, C.: Neuroanatomical focus for morphine and enkephalin induced hypermotility. Nature **265**, 645–646 (1977)

Rhodes, D. L., Leibeskind, J. C.: Analgesia from rostral brain stem stimulation in the rat. Brain Res. **143**, 521–532 (1978)

Slotnick, B. M., Leonard, C. M.: A stereotaxic atlas of the albino mouse forebrain. Washington, D. C.: U. S. Government Printing Office 1975

Tortella, F. C., Moreton, J. E., Khazan, N.: Electroencephalographic and behavioral effects of D-Ala^2 methionine enkephalinamide and morphine in the rat. J. Pharmacol. Exp. Ther. **206**, 636–642 (1978)

Urca, G., Frenk, H., Liebeskind, J. C., Taylor, A. N.: Morphine and enkephalin: Analgesic and epileptic properties. Science **197**, 83–86 (1977)

Vesell, E. S., Lang, C. M., White, W. J., Passananti, G. T., Tripp, S. L.: Hepatic drug metabolism in rats impairment in a dirty environment. Science **197**, 896–897 (1973)

Woodbury, D. M., Fingl, E.: Drugs effective in the therapy of the epilepsies. In: The pharmacological basis of therapeutics, L. S. Goodman, A. Gilman, eds., pp. New York: MacMillan 1975

Received December 14, 1978; Final Version June 5, 1979