J. Pharm. Pharmac., 1978, **30**, 310

COMMUNICATIONS

Analgesia and motor activity elicited by morphine and enkephalins in two inbred strains of mice

V. FRIGENI, F. BRUNO*, A. CARENZI, G. RACAGNI*†, V. SANTINI, *Zambon S.p.A., Research Laboratories, Bresso-Milan, Italy and *Institute of Pharmacology and Pharmacognosy, University of Milan, Milan, Italy*

Morphine injected intraperitoneally into mice produces analgesia and running activity that appear to be determined by genetic influence (Castellano & Oliverio, 1975). For example, C 57 BL/J (C 57) mice show high locomotor response when running behaviour is considered, whereas the same strain is less sensitive to the analgesic effect of morphine (Oliverio & Castellano, 1974) than DBA/2J (DBA) mice which have less locomotor response. Regional brain differences in the content of dopamine and acetylcholine have been suggested as being responsible for the opposite behavioural effects produced by morphine in the two strains of mice (Ebel, Hermetet & Mandel, 1973; Mandel, Ebel & others, 1973; Kempf, Greilsauer & others, 1974). To avoid the possibility that absorption and metabolism may play a role in determining the different responses to morphine in C 57 and DBA mice we used intracerebroventricular (i.c.v.) administration, to see whether results by this route confirmed those obtained with morphine given intraperitoneally. Moreover, the evidence for the existence in brain of an endogenous ligand with affinity for opiate receptors (Hughes, Smith & others, 1975) followed by the isolation of two pentapeptides methionine-enkephalin and leucine-enkephalin, prompted us to investigate whether the effects of intracerebroventricularly injected morphine could be reproduced by methionine (Met) enkephalin and the more potent D-Ala^2-Met-enkephalin.

Male DBA and C 57 mice (20–24 g) received intracerebroventricular injections as described by Haley & McCormick (1957). In all the experiments we compared the effects elicited by the opiates in the two inbred strains of mice with those produced by the same drugs in Swiss mice. Morphine hydrochloride, Met-enkephalin and D-Ala^2-Met-enkephalin were dissolved in pyrogen-free water and microinjected in a volume of 10 μl. Analgesia was measured by the hot plate method of Eddy, Fuhrmeister Touchberry & Lieberman (1950). The time in seconds from contact with the plate until a hind paw lick or jump occurred was recorded as the response latency. Morphine hydrochloride (0·5 μg/mouse), Met-enkephalin (50 μg/mouse) and D-Ala^2-Met-enkephalin (0·06 μg/mouse) were injected in doses that are approximately the ED50 for analgesia. The locomotor activity was measured with an Animex-activity meter (sensitivity 25—tuning set at 45) after a 30 min period of habituation. Morphine and Met-enkephalin were injected as previously described at a dose four-times higher than that used for analgesia. Since 200 μg of Met-enkephalin in 10 μl is close to maximum solubility, and we wished to demonstrate that the enkephalins do not alter locomotor activity in the strains of mice, we used the analogue D-Ala^2-Met-enkephalin, which can be administered at higher doses, at a dose ten-times higher than its analgesic ED50. Morphine-induced locomotor activity after intracerebroventricular administration of the drug show that the patterns of reactivity are different in DBA and C 57 mice (Fig. 1A). One h after injection, a four-fold average increase of motor activity was evident in C 57 mice in comparison with DBA mice. On the contrary, the analgesic response to morphine in DBA mice was greater than that obtained in C 57 mice (Fig. 1B). Both responses differ from that obtained in Swiss mice. Met-enkephalin, like morphine, was more potent in eliciting analgesia in DBA than in C 57 mice, while no motor response was observed in any of the strains investigated (Fig. 1D and 1C). Similar results were obtained using D-Ala^2-Met-enkephalin at the dose ten times higher than the corresponding ED50 for analgesia.

The use of inbred strains is a promising approach in the field of neuropharmacology. Genetic mechanisms seem to play an important role when the effects of morphine are assessed on the locomotor and analgesic behaviour in mice. Moreover, our data suggest that the strain differences in morphine sensitivity are not due to differences in absorption or metabolism, since the same effects are seen on analgesia and locomotor activity after intraventricular injection of the opiate.

Met-enkephalin resembles morphine in the strain specificity to the analgesic response. But, Met-enkephalin elicits no response in the two strains of mice when the locomotor activity is registered. The same results were obtained when D-Ala^2-Met-enkephalin was used at the dose 10 times higher than the ED50 for analgesia. These results indicate that morphine produces changes of the locomotor activity in the mouse either by mechanisms which differ from those mediating the analgesic

† Correspondence.

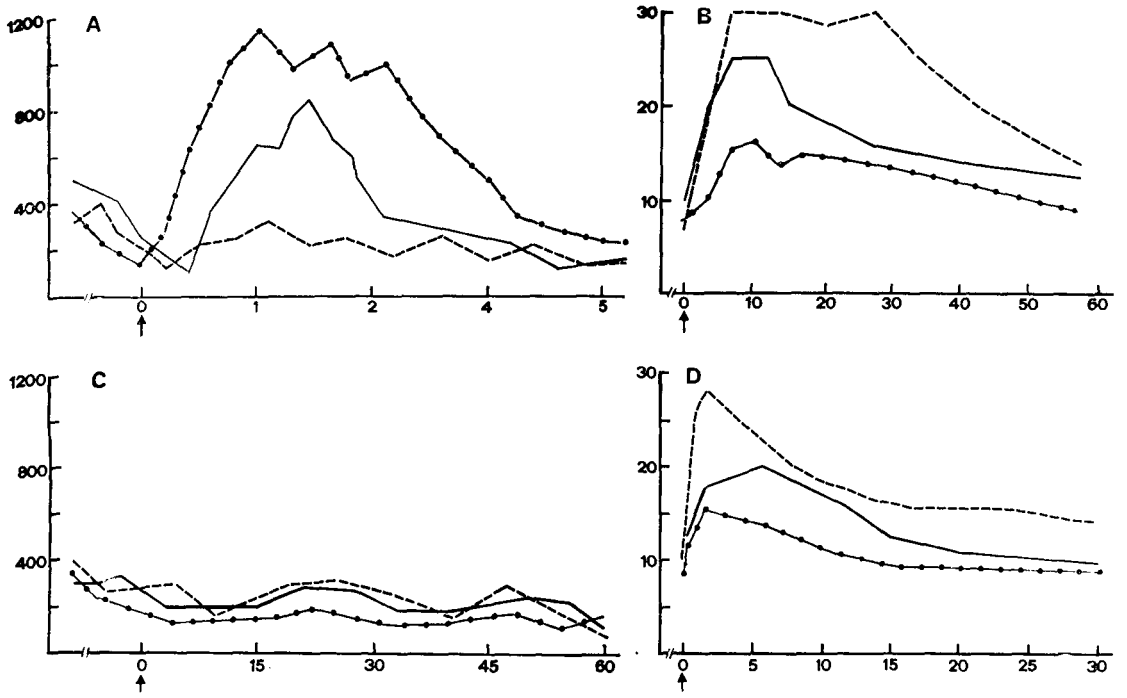

FIG. 1. Motor activity and analgesia elicited by morphine and Met-enkephalin in different strains of mice. Values are the average of at least four experiments (10 animals per experiment per dose). Left hand side: locomotor activity (counts per 5 min) induced by intracerebroventricular administration of morphine (2 μg) (A) and Met-enkephalin (200 μg) (C) (standard error <8%). Right hand side: antinociceptive response (s) induced by intracerebroventricular administration of morphine (0·5 μg) (B) and Met-enkephalin (50 μg) (D) (standard error <5%). Strains of mice: — Swiss; - - - DBA/2J; —●— C57 BL/6J. Abscissa: Time (min).

response or by the same mechanisms at different sites in the brain. In fact, the failure of Met-enkephalin to elicit hyperactivity in the C 57 strain implies either the existence of two different sites of action for morphine and enkephalin or the inolvement of different neurophysiological systems which might be responsible for the mediation of the analgesic and running behaviour.

Clouet & Ratner (1970) and Carenzi, Guidotti & others (1975) have reported that morphine is able to alter the synthesis and turnover of dopamine in the brain. Recent studies (Racagni, Oliverio & others, 1977) obtained studying the dynamics of striatal dopaminergic neurons in the two strains of mice after morphine have shown that this system is mainly involved in the locomotor activity elicited by morphine. Accordingly, the release of dopamine is increased only in C 57 mice (Trabucchi, Spano & others, 1976; Racagni & others, 1977).

November 29, 1977

REFERENCES

CARENZI, A., GUIDOTTI, A., REVUELTA, A. & COSTA, E. (1975). *J. Pharmac. exp. Ther.*, **194**, 311–318.

CASTELLANO, C. & OLIVERIO, A. (1975). *Psychopharmacologia*, **41**, 197–200.

CLOUET, D. H. & RATNER, M. (1970). *Science*, **168**, 854–856.

EBEL, A., HERMETET, J. C. & MANDEL, P. (1973). *Nature New Biol.*, **242**, 56–57.

EDDY, N. B., FUHRMEISTER TOUCHBERRY, C. & LIEBERMAN, J. E. (1950). *J. Pharmac. exp. Ther.*, **98**, 121–137.

HALEY, T. J. & MCCORMICK, W. G. (1957). *Br. J. Pharmac. Chemother.*, **12**, 12–15.

HUGHES, J., SMITH, T. W., KOSTERLITZ, H. W., FOTHERGILL, L. A., MORGAN, B. A. & MORRIS, H. R. (1975). *Nature*, **258**, 577–579.

KEMPF, E., GREILSAUER, J., MACK, G. & MANDEL, P. (1974). *Nature, New Biol.*, **247**, 483–485.

MANDEL, P., EBEL, A., HERMETET, J. C., BOVET, D. & OLIVERIO, A. (1973). *C.r. Acad. Sci. Paris*, **276**, 561–563.

OLIVERIO, A. & CASTELLANO, C. (1974). *Psychopharmacologia*, **39**, 13–22.

RACAGNI, G., OLIVERIO, A., BRUNO, F., MAGGI, A. & CATTABENI, F. (1977). *Adv. Biochem. Psychopharmac.*, **16**, 565–570.

TRABUCCHI, M., SPANO, P. F., RACAGNI, G. & OLIVERIO, A. (1976). *Brain Res.*, **114**, 536–540.