THE EFFECTS OF TWO PHENYLACETIC ACID DERIVATIVES ON THE ANALGESIC ACTION OF MORPHINE IN MICE

BY M. MEDAKOVIĆ AND B. BANIĆ

From the Department of Pharmacology, Medical Faculty, Belgrade, Yugoslavia

Received April 1, 1963

After intracerebral injection in mice, the analgesic effect of morphine depended on the dose. Two phenylacetic acid esters, CFT 1201 (diethylaminoethylphenyldiallyl acetate) and CFT 1208 (diethylaminoethylphenylallyl acetate), injected intraperitoneally, potentiated the analgesic action of 0·5 μg. of morphine, injected into the posterior portion of the brain 90 min. later. When the phenyl acetates were injected (50 μg.) into the brain, they potentiated the analgesic action of morphine, injected subcutaneously (10 mg./kg.) 80 min. later. The results show that the inhibitors of the hepatic microsomal enzymes may affect the action of analgesics by an action on the central nervous system.

SUBSTANCES which inhibit hepatic microsomal enzymes can potentiate and prolong the action of drugs being inactivated in the liver. Thus, carbon tetrachloride and some phenyl acetates potentiate and prolong the analgesic effect of morphine and similar analgesics. This phenomenon has been explained by an inhibitory action on the hepatic microsomal enzymes. However, some authors (Cook, Navis, Tonner and Fellows, 1953; Swinyard, Madsen and Goodman, 1954; Herken, Neubert and Timmler, 1959) suggested that the potentiating action of some of these agents (SKF 525-A and CFT 1201) was not only due to their action on the metabolic demethylation of drugs in the liver. Our recent experiments (Medaković and Banić, 1963) suggested also that carbon tetrachloride may increase the analgesic action of morphine not only by its action on the liver, but also by some mechanism which our data suggested might be an action on the central nervous system.

To further elucidate this possibility, experiments were arranged so as to minimise the influence of the inhibitors of the hepatic microsomal enzymes on the liver. This was achieved by using various routes of injection, and by combining these routes. Thus in some experiments morphine was injected in mice intracerebrally and the microsomal inhibitor intraperitoneally, while in other experiments the reverse order was followed. Further, the actions of two phenyl acetates on morphine analgesia have been studied. One of these, diethylaminoethylphenyldiallyl acetate (CFT 1201) is a potent inhibitor of hepatic microsomal enzymes, while the other, diethylaminoethylphenylallyl acetate (CFT 1208) has no such effects (Maibauer, Neubert and Rottka, 1958).

METHODS

Male white mice of approximately 20 g. were used. Analgesia was tested according to the method of Woolfe and Macdonald (1944). Each mouse in turn was placed on a hot plate (53°) and the reaction time

for the appearance of the paw licking reflex recorded. Groups of 10 mice were used and comprised only those animals which responded in a 10–15 sec. period. To prevent paw tissue damage in animals with analgesia, they were removed from the hot plate before this could occur. An arbitrary interval equivalent to double the control mean reaction time of each given group was selected for the purpose. The values were expressed on the graphs as percentages of the cut-off time. Thus the 50 per cent value in the graphs corresponds to the control mean reaction time of the corresponding group.

Intracerebral injections were according to Matthies and Schmidt (1961), into the hind brain, 2 mm. deep, with fine intradermal needles, approximately 3 mm. long. The drugs were dissolved in saline, to give a total volume of 0·01 ml. for the intracerebral injections.

The drugs used were: morphine hydrochloride, diethylaminoethylphenyldiallyl acetate hydrochloride (CFT 1201) and diethylaminoethylphenyallyl acetate hydrochloride (CFT 1208).

The results are presented graphically, but some of the animals were allowed to remain in contact with the hot plate until the cut-off time had expired, thus the analgesic effect as plotted is not exact and represents low values. Mean values which include two or more cut-off times have been represented in the graphs by open signs (circles, squares or triangles).

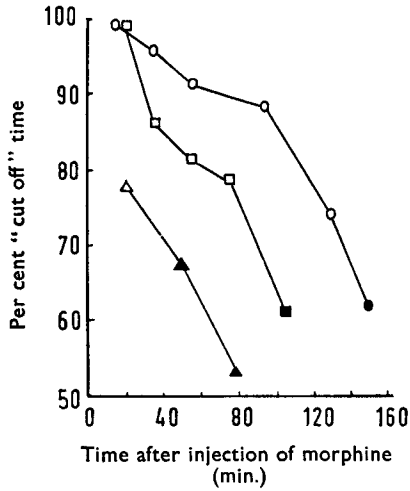

FIG. 1. Analgesic effect of three doses of morphine, injected intracerebrally. Triangles, 0·5 μg., squares, 1 μg. and circles, 2 μg. of morphine hydrochloride.

RESULTS

The action of intracerebrally injected morphine. Saline, injected into the brain, did not change the control mean reaction time. However, when morphine, dissolved in saline, was injected by the same route, the reaction times were prolonged (Fig. 1). Three groups of animals

were injected with 0·5, 1 and 2 μg. respectively. Fig. 1 shows that the effect of morphine was dependent on the dose injected.

The pH of the saline remained unchanged when morphine hydrochloride was dissolved either in concentrations used in these experiments or in much higher ones. Because of this, and also because of the dose: response relation, the prolongation of the reaction time was considered to be caused by the analgesic action of morphine.

Effects of compound CFT 1201 *and CFT* 1208 *on the action of intracerebrally injected morphine.* Compounds CFT 1201 and CFT 1208 respectively, were injected intraperitoneally (50 mg./kg.), 90 min. before morphine (0·5 μg.) was given intracerebrally. The animals in the control group received saline intraperitoneally. The results are in Fig. 2. Both

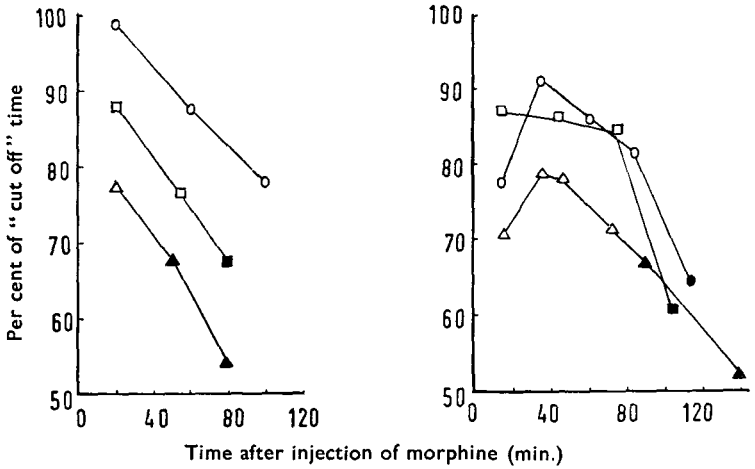

FIG. 2. The effects of compound CFT 1201 and 1208, injected intraperitoneally (50 mg./kg. of each) on the analgesic action of morphine, injected intracerebrally (0·5 μg.) 90 min. after the respective CFT compound. Triangles, morphine after saline; squares, morphine after CFT 1208; circles, morphine after CFT 1201.

FIG. 3. The effects of CFT 1201 and CFT 1208, injected intracerebrally (50 μg. each) on the analgesic action of morphine, injected subcutaneously (10 mg./kg.) 80 min. after the respective CFT compound. Triangles, morphine after saline; squares, morphine after CFT 1208; circles, morphine after CFT 1201.

CFT compounds were able to potentiate the analgesic action of morphine. Fig. 2 shows also that CFT 1201 had a higher potentiating activity than CFT 1208.

The control time before the injection of morphine revealed that compound CFT 1201 itself caused a moderate and short-lasting prolongation of the control mean reaction time. Neither compound CFT 1208 nor saline produced this effect. This effect of the compound CFT 1201 reached a maximum 15 min. after injection, but was insignificant when the morphine was injected.

The effect of the morphine (0·5 μg.) in animals which had previously received compound CFT 1201, was comparable to the effect of 2 μg. of morphine alone (Fig. 1). Thus the mean reaction time was prolonged to 99 per cent of the cut-off time 20 min. after the injection of morphine. At this time cut-off time was recorded in 8 out of 10 mice. In the control experiment (Fig. 1), 0·5 μg. of morphine produced a prolongation of the reaction time to 78 per cent of the cut-off time and only 4 out of 10 mice did not respond until the cut-off time.

As almost all mice receiving morphine after CFT 1201 did not respond until the cut-off time, the curve of the analgesic action shown in Fig. 2 is not the true effect, and it can be assumed that the potentiation of the effect of morphine is higher than is shown in Fig. 2.

In the mice receiving CFT 1208, the analgesic action of morphine was also potentiated, but less than by compound CFT 1201. Thus, only 5 out of 10 mice reached the cut-off time, and the mean reaction time was 89 per cent of the cut-off time. The potentiating effect of the compound CFT 1208 was also higher than presented in the graph. Consequently, the true difference between the potentiating activity of these two compounds cannot be clearly established.

Effect of intracerebrally injected CFT compounds on the analgesic action of subcutaneously injected morphine. In this experiment, CFT 1201 and CFT 1208 were injected intracerebrally (50 μg.) and then morphine was injected subcutaneously (10 mg./kg.) 80 min. later.

The first group of mice received CFT 1201. Immediately after, signs of central stimulation were observed. The animals appeared quiet, but, on touch, started running and some had convulsions. None died but those which convulsed were rejected.

Compound CFT 1201 caused moderate and reversible prolongation of the reaction time upon intracerebral injection. This effect was short-lasting and the reaction time returned to its starting control level long before morphine was injected.

The effect after morphine is shown in Fig. 3. Its action was potentiated by the previous injection of the compound CFT 1201. Potentiation was also obtained with the compound CFT 1208, both compounds apparently potentiating the effect of morphine to a similar degree. However, it can be assumed that the potentiating activity of CFT compounds was partly masked in this experiment also. Consequently, the relative potentiating activity of CFT compounds in this experimental design cannot be clearly established.

Discussion

The present experiments were aimed at throwing more light on the hypothesis that the so-called inhibitors of hepatic microsomal enzymes may affect the analgesic action of morphine by a mechanism independent from their action on the liver. Therefore, the routes of administration of drugs were chosen to minimise the influence of the metabolising capacity of the liver on the analgesic action of morphine.

It can be assumed that the analgesic action of morphine upon intracerebral injection was caused mainly by the direct effect of the drug; the concentration in the blood at this dose level having an insignificant effect, as 10 mg./kg. of morphine (approximately 200 μg. per mouse) has to be injected subcutaneously or intraperitoneally to obtain an effect comparable to that of 0·5 μg. of morphine injected into the brain.

The CFT compounds both intraperitoneally and intracerebrally potentiated the analgesic action of morphine. The potentiating intracerebral dose of the compound CFT 1201, was only 50 μg. while to inhibit hepatic microsomes, as much as 100 mg./kg. (approximately 2000 μg. per mouse) is usually injected intraperitoneally. It is therefore, improbable that after the intracerebral injection of 50 μg. of CFT 1201 the hepatic microsomes could be so strongly inhibited as to cause a delayed inactivation of morphine, and consequently a potentiation of its effect. That some other mechanism is probably involved is suggested also by the fact that both CFT 1201 and CFT 1208 potentiated the action of morphine. As already mentioned, compound CFT 1208 has been found not to inhibit hepatic microsomal enzymes in *in vitro* experiments (Maibauer and others, 1958).

When the CFT compounds were injected intraperitoneally, the effect of morphine was potentiated, and not prolonged. But, if CFT compounds acted by involving only the rate of the hepatic inactivation of morphine, the result would be prolongation rather than a potentiation.

Hence, the findings suggest that the so-called inhibitors of the hepatic microsomal enzymes may affect the action of analgesics not only by inhibiting their inactivation in the liver, but also by some other mechanism, a view already expressed by others (Cook and others, 1953; Swinyard and others, 1954; Herken and others, 1959; Herz, 1961). Thus, for example, that SKF 525-A, potentiated the analgesic action but did not affect the toxicity of morphine, could not be explained by the inhibition of the hepatic microsomes only (Cook and others, 1953). Similarly, SKF 525-A and CFT 1201 failed to potentiate the sedative stupor-inducing action of morphine (Herz, 1961). Further, carbon tetrachloride increased the analgesic action of morphine and codeine, but failed to affect the antidiuretic action of these drugs in our earlier experiments (Medaković and Banić, 1963). Hence, it can be assumed that these inhibitors of the hepatic microsomes can affect the action of analgesics by some more specific mechanism.

Carbon tetrachloride was shown to potentiate the inhibitory action of morphine on the twitch of the electrically stimulated isolated guinea-pig ileum (Medaković and Banić, 1963). Since this organ has been proposed as a suitable model for the analysis of actions of drugs which affect the central nervous system (Schaumann, Giovannini and Jochum, 1952; Paton, 1957), our finding suggested that carbon tetrachloride may potentiate the action of analgesics acting on the central nervous system. The present *in vivo* experiments indicated that the potentiating action of CFT compounds on the analgesic action of morphine was elicited by a mechanism involving the brain.

The question arises as to whether the potentiating effect shown by the CFT compounds had some connection with their ability to affect the enzymes. While their effect might possibly be a pharmacological synergism only, a connection between the effect of the CFT compounds and their ability to act upon enzymatic processes, is possible and is further suggested by the fact that CFT 1201 appeared to have a higher activity than CFT 1208.

Axelrod (1956) expressed the view that the metabolic changes, which the molecule of morphine undergoes in the liver, may be a model for the processes which occur at the receptors of the morphine action in the brain. In fact, the *in vivo* transformation of morphine in the brain has been demonstrated (Beckett, Casy and Harper, 1956; Milters, 1962), as well as a remarkable demethylation of pethidine by the rat brain tissue (Herken and others, 1959). It would be therefore interesting to examine the possible action of the inhibitors of hepatic microsomal enzymes on these and similar processes in the brain.

Acknowledgements.—CFT compounds were kindly supplied by Prof. Dr. H. Herken (Berlin) and Dr. Pflugk (Chemische Fabrik Tempelhof, Berlin).

References

Axelrod, J. (1956). *J. Pharmacol.*, **117**, 322–330.
Beckett, A. H., Casy, A. F. and Harper, N. J. (1956). *J. Pharm. Pharmacol.*, **8**, 874–883.
Cock, L., Navis, G. G., Tonner, L. and Fellows, E. G. (1953). *Fed. Proc.*, **12**, 299.
Herken, H., Neubert, D. and Timmler, R. (1959). *Arch. exp. Path. Pharmak.*, **237**, 319–333.
Herz, A. (1961). *Ibid.*, **241**, 236–253.
Maibauer, D., Neubert, D. and Rottka, H. (1958). *Ibid.*, **234**, 474–489.
Matthies, H. and Schmidt, J. (1961). *Ibid.*, **241**, 508–509.
Medaković, M. and Banić, B. (1963). *Acta med. Jug.* (in the press).
Milters, K. (1962). *Acta pharm. tox. Kbh.*, **19**, 235.
Paton, W. D. M. (1957). *Brit. J. Pharmacol.*, **12**, 119–127.
Schaumann, O., Giovannini, M. and Jochum, K. (1952). *Arch. exp. Path. Pharmak.*, **215**, 460–468.
Swinyard, E. A., Madsen, J. A. and Goodman, L. S. (1954). *J. Pharmacol.*, **111**, 54–63.
Woolfe, G. and Macdonald, A. D. (1944). *Ibid.*, **80**, 300–307.