J. Pharm. Pharmacol. 1988, 40: 434–437
Communicated January 26, 1988

Prodrug behaviour of nicotinoylmorphine esters

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Abstract—Morphine and its nicotinoyl esters, dinicotinoylmorphine (nicomorphine), 6-mononicotinoylmorphine (6-MNM) and 3-mononicotinoylmorphine (3-MNM) were tested in mice for central activity to obtain time-effect profiles of these compounds in rats. Two effects, analgesia with the hot plate test and locomotor stimulation in activity cages were measured and nicomorphine, 6-MNM and 3-MNM were found to have a faster onset of action compared with morphine. The effects of 3-MNM and morphine lasted longer than the effect of nicomorphine and 6-MNM. The prodrug behaviour of 3-MNM and nicomorphine for morphine and 6-MNM, respectively, is discussed.

Nicomorphine (Vilan), the 3,6-dinicotinoyl ester of morphine is currently used clinically for the induction of strong analgesia. Nicomorphine and other synthetic morphine-diesters like 3,6-diacetylmorphine (heroin) and 3,6-dibutanoylmorphine are much more lipophilic than morphine and hence penetrate the central nervous system much easier (Zirm et al 1959; Von Cube et al 1970; Andrew et al 1984). Morphine congeners with an esterified phenolic hydroxyl group like nicomorphine and 3-mononicotinoylmorphine (3-MNM) exhibit low opiate receptor affinity (Reden et al 1979; Lobbezoo et al 1980, 1982). As the 3-ester bond is rapidly hydrolysed and the 6-ester bond is more stable (Lindner & Raab 1981; Lindner & Semmelrock 1981; Lindner et al 1981; Lobbezoo et al 1982; van Rooy et al 1984), nicomorphine and 3-MNM can be considered as prodrugs for 6-mononicotinoylmorphine (6-MNM) and morphine respectively. The latter compounds are probably responsible for the analgesic effects of nicomorphine and 3-MNM, if the latter is analgesic at all. The present study is intended to follow the time course of action of nicomorphine, 3-MNM and 6-MNM compared with morphine in order to see the results of the interplay between the main determining factors of their effects, e.g. hydrolysis, lipophilicity, pK_a and opiate receptor affinity. Two different central opiate effects in mice were used, the analgesic effect measured with the hot plate test and locomotor stimulation measured in activity cages.

Materials and methods

Animals. Male mice, 20–25 g, were obtained from two suppliers. The experiments comparing the drugs at equal doses on a mg kg^{-1} base in the hot plate analgesia test and in locomotor activity were done with mice from Broekman Instituut, Someren, The Netherlands (strain Ha M/ICR, Swiss mice). Two additional experiments, comparing compounds at equimolar and equi-analgesic doses in the hot plate test, were performed at a later date and mice from Charles River, Sulzfeld, F. Repl. Germany (strain CD-1) were used. Before experiments mice were housed for 3–5 days in clear Perspex cages (40 × 24 × 14 cm; 25 mice per cage) with sawdust bedding material and under a controlled 12 h light dark cycle (lights on 0600 until 1800 h). The animals had free access to standard food pellets and water. Experiments were done between 0900 and 1600 h.

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Hot plate test. Analgesia was assessed in a hot plate test. The apparatus consisted of a copper plate surrounded by a transparent plastic cylinder 160 mm in diameter and 180 mm high. The plate was maintained at 56·0 ± 0·5°C. After drug administration mice were placed in a small Macrolon cage (5 mice per cage) until measurement of the response on the hot plate was made. Each mouse was placed on the hot plate and the time elapsed till the first reaction was recorded. Reactions were either licking a paw, flicking or raising a hindpaw or an attempt to jump out of the cylinder. If a mouse failed to react within 30 s it was removed from the plate and a latency of 30 s was recorded.

Each mouse was used once only, so that measurements on the various time points were obtained with different groups of mice and control groups.

Drug and vehicle treatments were made in a randomized sequence.

Locomotor activity. Locomotor activity was measured in transparent Macrolon cages (22 × 16 × 14 cm) the floor of which was covered with sawdust. Three infrared light beams, 2·5 cm above the cage floor, traversed the width of the cages. Two beams were 3 cm from the short sides of the cage and one beam traversed the middle. Two successive interruptions of adjacent light beams were recorded as one count. Mice were placed in a small plastic cage (5 mice per cage, 22 × 16 × 14 cm) after drug administration until measurement of locomotor activity. After placing one mouse per activity cage, counts were recorded for 10 min. Ten activity cages were situated in two soundproof ventilated boxes with dim light. Each mouse was used once only.

Drugs and injections. The drugs used were: nicomorphine HCl, 3-mononicotinoylmorphine HCl, 6-mononicotinoylmorphine HCl and morphine HCl (Diosynth, Oss, The Netherlands). All drugs were dissolved in 0·9% NaCl (saline). Freshly prepared drug solutions were injected subcutaneously into the neck in a dose-volume of 10 mL kg^{-1} body weight, control animals received an equivalent volume of saline.

Statistics. The Mann-Whitney U-test (two-tailed) was used to evaluate the significance of differences between groups of animals injected with different drugs and/or the control mice injected with saline.

Results

Analgesia in mice. Morphine, nicomorphine and both mononicotinoylmorphines injected subcutaneously in a dose of 10 mg kg^{-1} all induced an analgesic response, significantly different from control, 5 min after injection (Fig. 1). The peak effect of morphine was found approximately 10 min later in comparison with the other drugs. 60 min after injection, 6-MNM no longer showed significant analgesia, whereas morphine was still active at 120 min after injection. From the results in Fig. 1, a relative

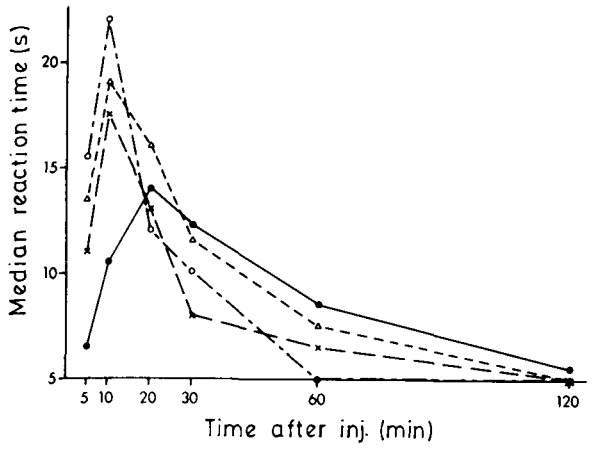

FIG. 1. Analgesic effect over time for morphine, nicomorphine, 3-MNM and 6-MNM with 10 mg kg^{-1} s.c. injections at t = 0 min. All values were different from placebo at $P < 0.05$ or smaller except for 6-MNM at 60 min and 120 min and nicomorphine and 3-MNM at 120 min. The following differences had chance values < 0·05 with Mann-Whitney U-tests: morphine vs each of the other drugs at 5 and 10 min after injection, morphine vs nicomorphine at 30 min and nicomorphine vs 6-MNM at 5 min after injection. n = 20 for placebo groups and the morphine group at 30 min, n = 10 for all other groups. For reasons of clarity ranges are omitted and scale starts at 5 s, below which are placebo values. Interquartile ranges for morphine were 5–8; 10–17; 12–17; 10–15; 6–10; 5–8 for 5 to 120 min time points, for nicomorphine 8–13; 11–20; 10–18; 7–10; 5–10; 5–6, respectively; for 3-MNM 9–17; 14–27; 14–19; 9–16; 5–10; 4–6, respectively, and for 6-MNM 14–18; 15–26; 10–14; 8–10; 5–7; 5–6, respectively. The interquartile ranges for the placebos were 4–6 or smaller. Key: (●) morphine HCl, 10 mg kg^{-1} s.c.; (×) 3,6-dinicotinoyl morphine HCl, 10 mg kg^{-1} s.c.; (△) 3-nicotinoyl morphine HCl, 10 mg/kg s.c.; (○) 6-nicotinoyl morphine HCl, 10 mg/kg s.c.

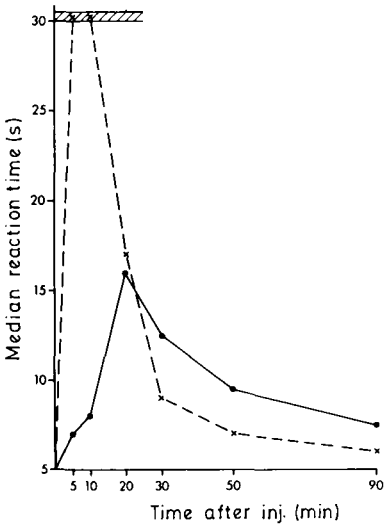

FIG. 2. Analgesic effect over time of 20 μmol kg^{-1} 3,6-DNM in comparison with 20 μmol kg^{-1} morphine. Scale starts at 5 s below which are placebo results. Values above 5 s reaction time have chance values < 0·05 except for the result with 3,6-DNM at 90 min. At 5 and 10 min after injection effect of morphine and 3,6-DNM are different with $P < 0.002$. Interquartile ranges for morphine (at 5–90 min time points): 6–9; 7–10; 15–20; 9–14; 8–12 and 6–9; for 3,6-DNM: 30–30; 16–30; 16–8; 7–12; 6–10 and 5–7. The interquartile ranges for the placebos were 3–5, 4–6 or smaller. We used 10 animals per time point for drug-treated groups and 20 animals for placebo-treated groups. Key: (●) morphine HCl 6·4 mg kg^{-1} = 20 μmol kg^{-1} s.c.; (×) 3,6-dinicotinoyl morphine HCl 10·6 mg kg^{-1} = 20 μmol kg^{-1} s.c.

order of analgesic activity at this dose can be discerned. The order of 6-MNM > nicomorphine > 3-MNM > morphine during the first 10 min after injection. This was reversed 30 min after injection to: morphine ≈ 3-MNM > 6-MNM > nicomorphine. This pattern was reproduced in another experiment with 10 mg kg^{-1} with measurements 5,10,15,20 and 30 min after injection (data not shown here).

In two additional experiments this pattern was more specifically confirmed by comparing 3,6-DNM with morphine in equimolar doses and 3-MNM with morphine in approximately equianalgesic doses. In Fig. 2 results are depicted of the effect of 3,6-DNM and morphine at 20 μmol kg^{-1}. The maximum effect of 3,6-DNM is about two times higher than the maximum effect of morphine. Nevertheless, morphine, 30 min and longer after injection, tended to be more potent than 3,6-DNM. In Fig. 3 the effect of 5 mg kg^{-1} 3-MNM is given in comparison to the effect of 10 mg kg^{-1} morphine. It can be seen that the same maximal analgesic effect was obtained, but that 3-MNM had a faster onset of action. The effect of morphine lasted longer.

Locomotor activity in mice. Morphine, nicomorphine, 3-MNM and 6-MNM injected in a dose of 5 mg kg^{-1} s.c., all stimulated locomotor activity in mice (Fig. 4). Morphine elicited enhanced locomotor activity 30–40 min after injection, whereas nicomorphine, 3-MNM and 6-MNM already showed significant locomotor stimulation 5–15 min after injection. We found no locomotor stimulation 30–40 min after injection with nicomorphine and 6-MNM. The locomotor activity response to morphine terminated 60–70 min after injection, whereas there was still a significant locomotor stimulation induced by 3-MNM at

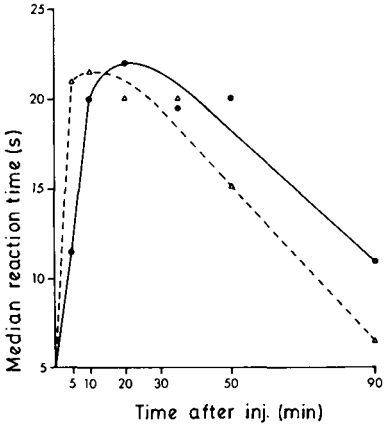

FIG. 3. Analgesic effect over time of 5 mg kg^{-1} 3-MNM in comparison with 10 mg kg^{-1} morphine. At 5 min after injection this dose of 3-MNM is more potent than 10 mg kg^{-1} morphine ($P < 0.002$). At 90 min after injection 5 mg kg^{-1} 3-MNM is less potent than 10 mg kg^{-1} morphine ($P < 0.05$). Other differences are not statistically significant. No placebo-treated group was included in this experiment. Interquartile ranges for morphine (at 5–90 min time points): 9–14; 15–22; 20–30; 18–21; 16–30 and 10–12; for 3-MNM: 18–26; 17–30; 14–24; 15–23; 11–21 and 5–8. We used 10 animals in each group. Key: (●) morphine HCl, 10 mg kg^{-1} = 31 μmol kg^{-1} s.c.; (△) 3-nicotinoyl morphine HCl, 5 mg kg^{-1} = 11·7 μmol kg^{-1} s.c.

120–130 min after injection. A higher dose of morphine (10 mg kg^{-1}) stimulated activity for longer than 70 min, whereas the effect of 10 mg kg^{-1} nicomorphine stopped at 60–70 min after injection (data not shown).

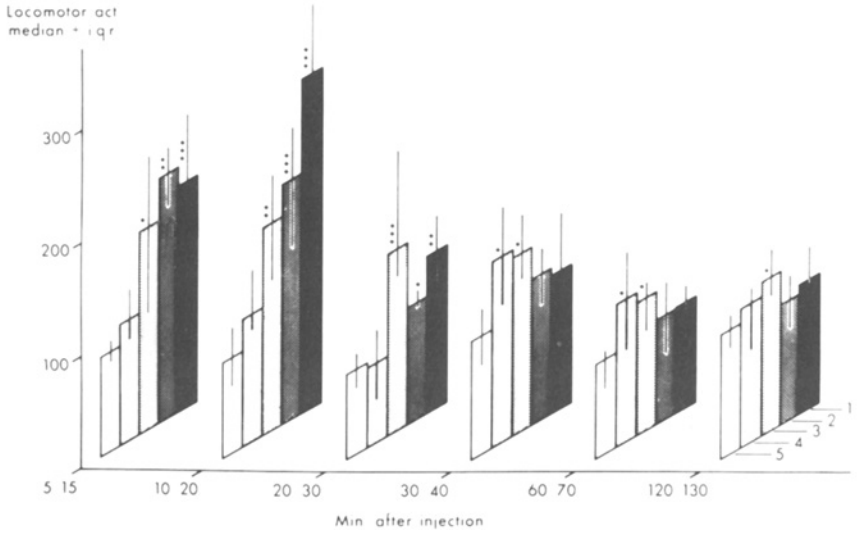

FIG. 4. Locomotor activity in mice. 10 min scores (median + interquartile range; n = 10 for treatment groups and placebo) at various times after s.c. injection (t = 0 min) of 5 mg kg^{-1} of the indicated drugs (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$, drug treatment vs placebo). Key: 1 = 6-MNM, 2 = nicomorphine, 3 = 3-MNM, 4 = morphine, 5 = placebo.

Discussion

The rapid onset and peak effect of nicomorphine, 3-MNM and 6-MNM in comparison with morphine in both locomotor activity and the hot plate test probably reflects the difference in brain penetrability together with a rapid hydrolysis of nicomorphine and 3-MNM in the CNS into compounds with high opiate receptor affinity. The easy brain penetration of the esterified analogues is due to their higher lipophilicity (log octanol–water partition coefficients 0·45, 1·44 and 2·17 for morphine, 6-MNM and nicomorphine, respectively) and their lower pK_a values [at 37°C: 7·83, 7·39, 7·72 and 7·63 for morphine, DNM, 6-MNM and 3-MNM, respectively] (Hull 1983). Fundamental to this explanation is that a simple relationship between brain concentration of active opiate and pharmacological effect exists. Although there is no unanimity on this subject (Dahlström et al 1978; Plomp et al 1981; Levine et al 1983), two reports (Patrick et al 1975; Hipps et al 1976) describe a good correlation between brain concentration of morphine and analgesic activity in mice after s.c. injection of morphine (8–10 mg kg^{-1}). Both studies demonstrate a peak in brain morphine levels about 30 min after injection accompanied by a maximal analgesic effect. The hot plate analgesia test appears to be more sensitive for the detection of central opiate activity because locomotor activity is only significantly enhanced 30–70 min after injection of 10 mg kg^{-1} morphine, whereas statistically significant analgesia in the hot plate test occurs from 5 up to and including 120 min after injection of 10 mg kg^{-1} morphine.

Concerning the duration of the central opiate effects it can be concluded that there is a difference between nicomorphine and 6-MNM on the one hand and morphine and 3-MNM on the other hand. As with the onset of action the results on the duration of action can be identified with the three factors: (i) brain penetration, (ii) opiate receptor affinity and (iii) rapid hydrolysis of the 3-ester bond. Our explanation is that nicomorphine rapidly enters the brain and is hydrolyzed to 6-MNM (Lindner et al 1981). 6-MNM has a high affinity for the opiate receptor (Lobbezoo et al 1982), but leaves the brain easily because it is mostly in neutral form and has a high lipophilicity. 3-MNM also rapidly enters the brain where it is hydrolysed to morphine, but morphine leaves the brain more slowly because of its low lipophilicity and higher pK_a. Systematically administered morphine results in slow onset and longer duration of action, due to low lipophilicity and a larger percentage of molecules in the charged hydrogenated form (pK_a above physiological pH). The greater analgesic activity of 3-MNM compared with morphine is probably the result of a more efficient transport of 3-MNM and hence higher concentration of morphine in the central nervous system after local hydrolyses of 3-MNM. Umans & Inturrisi (1981) conclude in their studies with diacetylmorphine (heroin), 6-monoacetylmorphine (6-MAM) and morphine that heroin and 6-MAM exhibit virtually identical time-effect profiles, measuring antinociception after s.c. injections of these drugs in mice. Both drugs reached their peak effect more rapidly than morphine while their duration of action was shorter compared with morphine. We find that it is in the 3-mono-ester forms of morphine where a rapid onset of action is combined with a long duration of action.

The authors thank Mesdames B. v.d. Heyden and G. Vos for their contribution.

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J. Pharm. Pharmacol. 1988, 40: 437–438
Communicated November 30, 1987

The stable prostacyclin-analogue, iloprost, unlike prostanoids and leukotrienes, potently stimulates cyclic adenosine monophosphate synthesis of primary astroglial cell cultures

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Abstract—The effect of different eicosanoids on adenosine-3', 5'-cyclic-monophosphate (cAMP) accumulation in primary astroglial cell cultures prepared from newborn rat brain was studied. The stable prostacyclin-analogue, iloprost, effectively stimulated cAMP synthesis in a concentratioin-dependent, saturable manner, the EC50 being about 3×10^{-8} M. Prostaglandin (PG) E_2 was less potent, without reaching plateau even at 10^{-5} M. Prostaglandins D_2 and $F_{2\alpha}$, and the stable thromboxane A_2-analogue, U 46619, as well as leukotrienes (LT) B_4, C_4, D_4 and E_4 were not effective and did not attenuate basal or isoprenaline (10^{-8} M)-stimulated astroglial cAMP formation. This is the first indication for the existence of a prostacyclin receptor coupled positively to the adenylate cyclase in astrocytes. Other eicosanoids are unlikely to be involved in receptor-mediated regulation of astroglial cAMP levels.

Prostanoids and leukotrienes are biologically active oxidation products of arachidonic acid (Needleman et al 1986). The synthesis of prostanoids (Wolfe 1982) and LTs (Simmet et al 1987) by brain tissue has been described. Except for prostacyclin, which is mainly of vascular origin (Wolfe 1982), cerebral prostanoids originate predominantly from mature astrocytes (Seregi et al 1987), while the cellular source of LTs in the brain remains to be elucidated. Prostanoids possess central effects of phsyiological relevance (Shimizu et al 1979; Wolfe 1982; Hertting et al 1985). Little is known however about the role of LTs in the brain (Moskowitz et al 1984). There is a growing interest concerning the biological response of astrocytes to putative signal-transducing substances (Murphy & Pearce 1987). Therefore, in this study, we have investigated the effects of prostanoids and LTs on receptor-coupled adenylate cyclase activity of astrocytes. As a model, primary astroglial cells cultures prepared from neonatal rat brain hemispheres were used.

Methods and materials

Details of the preparation and culture conditions as well as the morphological and immunocytochemical characterization of the cultures have been described recently (Keller et al 1985).

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Fourteen day old cultures were studied. For experimental incubations cells were washed in 0·01 M Na-phosphate buffer (pH 7·4) containing 0·15 M NaCl and 0·1% (w/v) glucose (solution A), and preincubated for 15 min at 37°C in 1 mL solution A supplemented with 10^{-5} M indomethacin and 5×10^{-4} M isobutylmethyl xanthine (solution B). Incubations were carried our for 3 min at 37°C in 1 mL solution B in the presence of the actual compounds to be tested. The inhibitors examined were present both during preincubation and incubation. The reaction was stopped by adding 0·1 mL 1·5 M $HClO_4$, and cultures were allowed to stand for 30 min at 4°C. Supernatants were transferred into plastic vials, neutralized by excess $CaCO_3$ (Tihon et al 1977) and centrifuged (2000 $g \times 10$ min, 4°C). Cyclic AMP concentrations were estimated from the resultant supernatants by a specific radioimmunoassay as described previously (Ortmann 1978). Protein was determined according to Lowry et al (1951).

Iloprost was kindly donated by Schering A.G. (Berlin, FRG), Leukotrienes C_4 and E_4 were the generous gift of Hoechst A.G. (Frankfurt, FRG). U 46619 [(15 S)-hydroxy-11α, 9α (epoxy methano) prosta-52, 13E-dienoic acid] was kindly supplied by Dr Th. Simmet. All other substances and chemicals were from commercial sources.

Results and discussion

Fig. 1. shows the effect of various prostanoids or their mimetics on basal cAMP formation of astrocytes.

In contrast to a human astrocytoma cell-line, where prostacyclin (PGI_2) proved to be a very weak stimulant of cAMP synthesis (Ortmann 1978), in the primary astroglial cell cultures, the stable PGI_2-mimetic agent iloprost (Schrör et al 1981) was the most effective substance in increasing cAMP accumulation. The effect of iloprost was concentration-dependent and reached plateau at 10^{-6} M. The EC50 value was about 3×10^{-8} M, which is close to that obtained for PGI_2 on platelet adenylate cyclase (Gorman et al 1977). These results strongly suggest that cultured astrocytes possess a prostacyclin receptor positively coupled to their adenylate cyclase activity. It may be of special interest since