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Stereospecific inhibition of oxotremorine-induced antinociception by (+)-isomers of opioid antagonists: comparison with opioid receptor agonists

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The antagonistic effects of the two benzomorphan opioid antagonists, Mr-1452 and Mr-2266 and their respective (+)-isomers Mr-1453 and Mr-2267 upon morphine, ethylketocyclazocine (EKC), D-ala^2-D-leu^5-enkephalinamide (BW 180-C) and oxotremorine (OTMN) antinociceptive activity in mice were investigated. Pretreatment with either Mr-1452 (2·0 mg kg^{-1} i.p.) or Mr-2266 (2·0 mg kg^{-1} i.p.) significantly antagonized the antinociceptive effects of the three opioid agonists in the hot plate test, but were ineffective against OTMN, which in contrast was antagonized by the (+)-isomers. Interaction between the antagonists and submaximal analgesic doses of the opioids or OTMN produced similar results in the tail immersion assay. However, the effect of Mr-2267 on OTMN was biphasic and this contrasted with Mr-1453 which produced consistent and graded antagonism.

The relationship between opioid and cholinergic analgesia is not fully understood. Harris et al (1970) demonstrated the antagonistic properties of the opioid antagonist (±)-naloxone against oxotremorine (OTMN)-induced antinociception in mice. Recently Pedigo et al (1975) reported that centrally administered acetylcholine (ACh) in mice produced a dose-dependent antinociceptive activity which was inhibited by both isomers of pentazocine and cyclazocine. In this respect the (+)-isomers were several times more potent than their respective (−)-isomers. More recently, Koehn et al (1980) examined the effects of the opioid antagonist benzomorphan Mr-2266 and its (+)-isomer Mr-2267 on the antinociceptive effect of the irreversible cholinesterase inhibitor diisopropylphosphofluoridate (DFP) in the rat hot plate test. They demonstrated that DFP antinociception was antagonized by Mr-2266 at an opiate specific dose and to a lesser extent inhibited by Mr-2267. Accordingly, the question arises whether OTMN, which, at doses which may stimulate central sites is believed to act exclusively at muscarinic receptors (Cho et al 1962), displays any stereospecific sensitivity towards opioid antagonists. Possible OTMN sensitivity has been compared with reputed selective opioid receptor agonists as recently hypothesized by Martin et al (1976) and Lord et al (1977).

MATERIALS AND METHODS

Male albino mice, GB1 variants of an ICI derived strain, 18–20 g, were allowed free access to a standard cube diet and water, both being withdrawn 2 h before the first experimental results were recorded. All experiments were carried out between 10.00 and 13.00 h at a temperature of 22 ± 0·5 °C.

Nociceptive sensitivity was determined using the hot plate (55 °C) method of Woolfe & McDonald (1944) and by the tail immersion test (48 °C) of Sewell & Spencer (1975). In the hot plate test nociceptive reaction times (s) were measured 30 min after subcutaneous (s.c.) injections of OTMN, morphine, ethylketocyclazocine (EKC) or intracerebroventicular (i.c.v.) injections of BW 180-C.

In the tail immersion test, response latencies were determined (s) when the stimulus was applied to each animal at 20 min intervals for 120 min. As in the hot plate, a cut off time of 20 s was imposed during the measurement of response latencies. From the time course of the effect of each drug an index termed '% antinociceptive effect' was calculated as follows: % antinociceptive effect = ((T − C/T) × 100, where T and C represent the area under the curve [integral of the effect (reaction time in s) vs experimental duration in min)] for test drug and vehicle-treated groups respectively. In all experiments involving interaction between agents, animals were pretreated with doses of benzomorphan 30 min before the test drug.

Statistical analysis of data. Statistical significance of differences between group means was assessed by calculating Student's *t*-(two tailed) and significance was assumed at the $P < 0{\cdot}01$ level.

Drugs. The drugs used were: oxotremorine sesquifumarate (Aldrich), morphine HCl (Macarthys),

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ethylketocyclazocine (Sterling-Winthrop), BW 180-C (D-ala^2-D-leu^5-enkephalinamide) (Wellcome Laboratories), Mr-1452, Mr-1453 (respective (−)- and (+)-isomers of 5,9-dimethyl-3(furylmethyl)-2′-hydroxy-6,7-benzomorphan), Mr-2266 and Mr-2267 (respective (−)- and (+)-isomers of 5,9-diethyl-3-(furylmethyl)-2′-hydroxy-6,7-benzomorphan) (Boehringer Ingelheim). Mr-2266 and Mr-2267 were dissolved in 1·0 ml 5% w/v (±)-tartaric acid and the volume was made up with 0·9% NaCl (saline), the final pH being adjusted to 5·6. All other drugs were dissolved in normal saline.

RESULTS

Effect of benzomorphan pretreatment on morphine, EKC, BW 180-C and OTMN antinociception in the tail immersion test

The effect of 30 min pretreatment with various doses of Mr-1452, Mr-1453, Mr-2266 and Mr-2267 on the antinocicpetive activity produced by submaximal doses of morphine (2·5 mg kg^{-1} s.c.), EKC (0·5 mg kg^{-1} s.c.), BW 180-C (1·0 μg/animal i.c.v.) and OTMN (50 μg kg^{-1} s.c.) are shown in Figs 1, 2, 3 and 4. Mr-1452 and Mr-2266 at doses ranging from 0·5 to

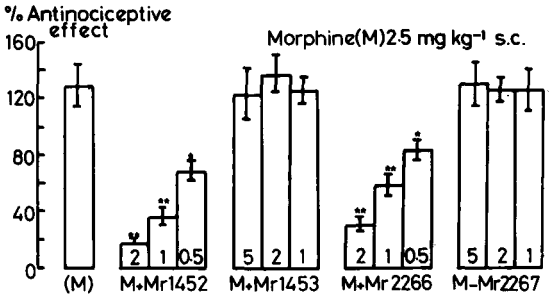

FIG. 1. Effect of either (−)-(Mr-1452 and Mr-2266) or (+)-(Mr-1453 and Mr-2267) isomers of benzomorphans injected i.p. 30 min before morphine (2·5 mg kg^{-1} s.c.) in tail immersion test. Numbers inside bars indicate various doses of benzomorphans. Results are mean % antinociceptive effect ± s.e. (8–10 animals). $^*P < 0.01$; $^{**}P < 0.001$ vs control responses.

2·0 mg kg^{-1} (i.p.) produced a dose-dependent significant reduction in the activities of morphine (Fig. 1), EKC (Fig. 2) and BW 180-C (Fig. 3). This contrasted with their effect on OTMN-induced antinociception which was not significantly modified by either Mr-1452 or Mr-2266 (Fig. 4). However a dose-dependent and significant reduction in OTMN activity was obtained following Mr-1453 (0·25, 0·5, 1·0 and 5·0 mg kg^{-1}) pretreatment. Unlike Mr-1453, pretreatment with Mr-2267 produced a biphasic effect on OTMN activity in that attenuation occurred

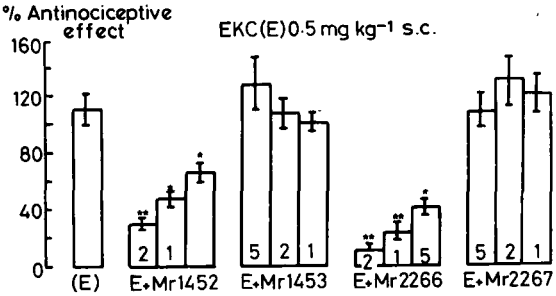

FIG. 2. Effect of either (−)-(Mr-1452 and Mr-2266) or (+)-(Mr-1453 and Mr-2267) isomers of benzomorphans injected i.p. 30 min before EKC (0·5 mg kg^{-1} s.c.) in tail immersion test. Numbers inside bars indicate various doses of benzomorphans. Results are mean % antinociceptive effect ± s.e. (8–10 animals). $^*P < 0.01$; $^{**}P < 0.001$ vs control responses.

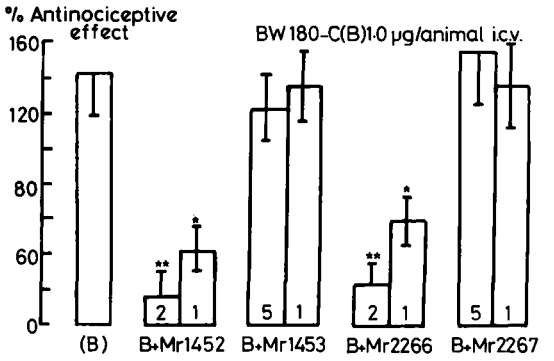

FIG. 3. Effect of either (−)-(Mr-1452 and Mr-2266) or (+)-(Mr-1453 and Mr-2267)-isomers of benzomorphans injected i.p. 30 min before BW 180-C (1·0 μg/animal i.c.v.) in tail immersion test. Numbers inside bars indicate various doses of benzomorphans. Results are mean % antinociceptive effect ± s.e. (8–10 animals). $^*P < 0.01$; $^{**}P < 0.001$ vs control responses.

at a dose of 5·0 mg kg^{-1} and lower, whilst the higher dose (10·0 mg kg^{-1}) produced a slight but significant ($P > 0.01$) enhancement and the intermediate dose (7·5 mg kg^{-1}) produced no significant change (Fig. 4). Neither Mr-1453 nor Mr-2267 pretreatment appeared to produce any significant change in the activity of the opioids.

Effect of benzomorphan pretreatment on morphine, EKC, BW 180-C and OTMN antinociception in the hot plate test

The effects of pretreatment with Mr-1452, Mr-1453, Mr-2266 and Mr-2267 on the dose-response lines of either the opioids or OTMN are illustrated in Figs 5 and 6. Thirty min pretreatment with either Mr-1452

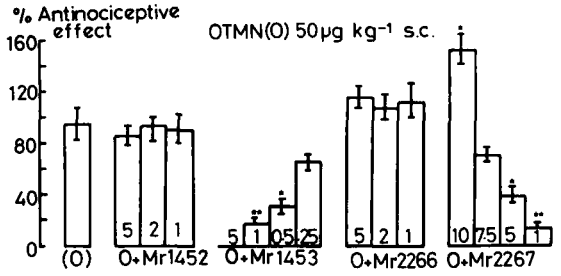

FIG. 4. Effect of either (−)-(Mr-1452 and Mr-2266) or (+)-(Mr-1453 and Mr-2267) isomers of benzomorphans injected i.p. 30 min before OTMN (50 μg kg^{-1} s.c.) in tail immersion test. Numbers inside bars indicate various doses of benzomorphans. Results are mean % antinociceptive effect ± s.e. (8–10 animals). $^{*}P < 0.01$; $^{**}P < 0.001$ vs control responses.

(2·0 mg kg^{-1} i.p.) or Mr-2266 (2·0 mg kg^{-1} i.p.) significantly lowered nociceptive latencies, decreasing both the peak intensity and duration of the antinociceptive responses produced by BW 180-C, morphine or EKC. This was reflected by a significant rightward shift in the dose response lines of the opioids which were not affected by the (+)-isomers

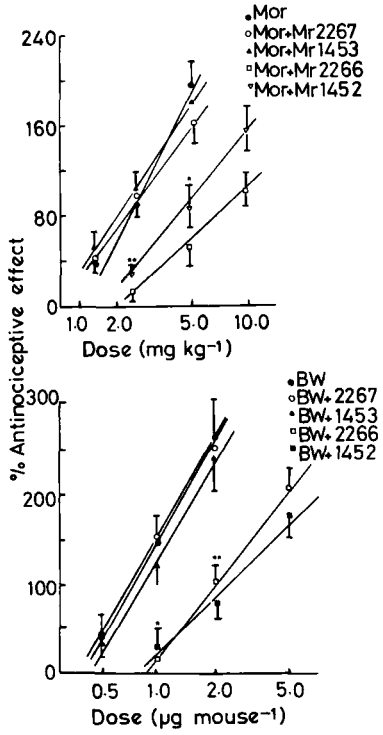

FIG. 5. Dose-response regression lines for either morphine (MOR) or BW 180C (BW) alone or in combination (30 min pretreatment) with either Mr-1452, Mr-1453, Mr-2266 or Mr-2267 in the hot plate test. $^{*}P < 0.01$; $^{**}P < 0.001$.

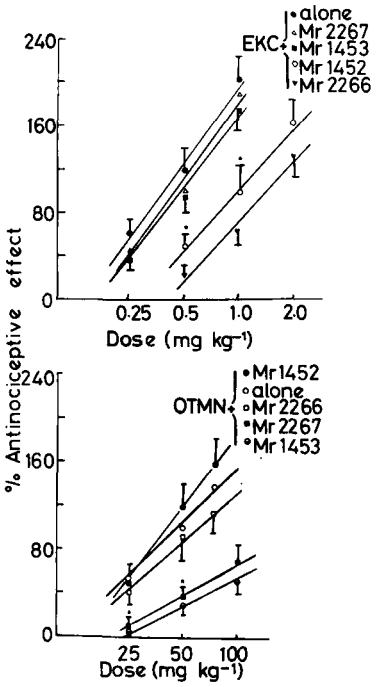

FIG. 6. Dose-response regression lines for either ethylketocyclazocine (EKC) or oxotremorine (OTMN) alone or in combination (30 min pretreatment) with either Mr-1452, Mr-1453, Mr-2266 or Mr-2267 in the hot plate test. $^{*}P < 0.01$.

and it contrasted with OTMN activity which did not appear to be significantly changed by Mr-1452 or Mr-2266. However, after 30 min pretreatment with either Mr-1453 (1·0 mg kg^{-1} i.p.) or MR-2267 (2·0 mg kg^{-1} i.p.) there was a marked rightward shift in the dose response line for OTMN indicating an antagonism in this latter case.

Unlike the (+)-isomers, neither Mr-1452 nor Mr-2266 appeared to produce any significant change in OTMN antinociception. Similarly, the activity of the opioids did not appear to be significantly modified by Mr-1453 nor Mr-2267 pretreatment at the above doses.

DISCUSSION

The present investigations demonstrate that, at least in mice, OTMN and opioid analgesics exhibit a reciprocal stereospecific sensitivity towards opioid antagonists. This is partly compatible with recent observations of Pedigo et al (1975) who showed that (+)-isomers of some opioid partial agonists, namely pentazocine and cyclazocine, were more potent than their respective (−)-isomers in blocking ACh-induced antinociception in the mice tail flick assay.

However in the present study, pretreatment with the true opioid antagonists Mr-1452 and Mr-2266 did not appear to produce any significant inhibition in OTMN-induced antinociception. This slightly divergent observation could be attributed to the fact that those authors used partial agonists to reverse ACh activity in contrast to the true antagonists we used. In addition ACh with its well known mixed muscarinic and nicotinic properties may produce its antinociception via either combined or separate activation of muscarinic or nicotinic mechanisms. This is not the case with OTMN which is devoid of in vivo nicotinic activity (see introduction). However, this combined muscarinic/nicotinic interpretation is not tenable since the specific nicotinic receptor blocker mecamylamine was reported to be ineffective against ACh-induced antinociception (Dewey et al 1976). Another possibility is that OTMN may produce its antinociceptive activity via different subpopulations of muscarinic receptors or even a non-cholinergic mechanism. Several lines of evidence are in favour of this hypothesis. Firstly, recent studies in our laboratories have shown that whilst Mr-1453 and Mr-2267 antagonized OTMN-induced antinociception, such antagonism could not be demonstrated for OTMN's other central effects such as tremor and hypothermia (Ben-Sreti et al 1982) which are thought to be mediated by cholinergic mechanisms (Crossland & Slater 1968; Haubrich et al 1972). Secondly, Waterfield & Kosterlitz (1975) reported that (+)-isomers of narcotic antagonists were inactive in modifying electrically evoked ACh release from myenteric plexus longitudinal muscle of the guinea-pig ileum. In this context it should be noted that despite the fact that atropine was found to be effective against most of OTMN's central effects, its inhibition of OTMN antinociceptive activity could be due to atropine's inherent hyperalgesic activity, thus leading to a functional antagonism (Ben-Sreti & Sewell 1981). Thirdly, it has been demonstrated recently that tolerance to the tremorgenic and hypothermic activity of tremorine (precursor of OTMN) is readily abolished by increasing doses of OTMN, whilst tolerance to antinociception is insurmountable (Maayani et al 1977; György 1979). Further, Slater (1974) reported that OTMN-induced antinociception rather than tremor or hypothermia is abolished by 5-hydroxydopamine pretreatment. Finally, the β-adrenoceptor antagonist propranolol which significantly decreases total brain ACh (Khana & Madan 1973) was found to inhibit OTMN-induced tremor without interfering with OTMN antinociception (Barar & Madan 1976). Consequently, OTMN may well produce its antinociception through a neuronal substrate which may or may not indirectly involve a cholinergic system. This substrate appears to be sensitive to the (+)-isomers of narcotic antagonists.

Our results are also difficult to reconcile with those of Koehn et al (1980) who reported DFP-induced antinociception in the rat to be antagonized by Mr-2266, whilst the (+)-isomer Mr-2267 produced only slight attenuation. This difference in finding may be due to the fact that a 5-hydroxytryptaminergic system may mediate pain mechanisms (Messing & Lytle 1977) and play a role in tremorine (Sethy et al 1971) and presumably OTMN antinociception in mice, whilst there is no evidence to implicate this system in cholinergic analgesia in the rat (Paalzow & Paalzow 1975; Koehn et al 1978). It may also be possible that DFP-induced antinociception is indirectly mediated via an endogenous opioid system (Koehn et al 1980) which is not shared by OTMN, despite the fact that the latter authors were unable to show cross tolerance between DFP and morphine, which may be expected if they share a common mechanism. Our data suggests that even though there may be a stereochemical similarity between opioid and cholinergic receptors as has been recently hypothesized (Pauling 1975), OTMN antinociceptive activity appears to be independent of endogenous opioid systems, since it remained unmodified by Mr-1452 or Mr-2266 pretreatment. Finally, our observations that the effect of Mr-2267 on OTMN antinociceptive activity in the tail immersion test was biphasic may be attributed to the fact that Mr-2267 at doses of 10·0 mg kg^{-1} and higher increased nociceptive latencies in the tail immersion test (unpublished data). Thus the apparent increase in OTMN activity produced by Mr-2267 (10·0 mg kg^{-1}) could be the result of summation of effects rather than true potentiation.

In conclusion, evidence has been presented in favour of separate analgesic mechanisms for OTMN and opioid analgesics. However, it remains to be determined whether the effects of other non-opioid and non-cholinergic analgesics could be modulated in a similar way to OTMN.

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