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In-vivo studies with the opioid antagonist, 16-methylcyprenorphine

PHILLIP J. BIRCH, ANN G. HAYES, MICHAEL J. SHEEHAN, MICHAEL B. TYERS, *Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DJ, UK*

Abstract—The effect of the opioid antagonist, 16-methylcyprenorphine (RX8008M), on the antinociceptive action of the μ-selective agonist, morphine, and the κ-selective agonist, U50488H, has been investigated in the mouse abdominal constriction test. RX8008M produced a dose-dependent antagonism of the antinociceptive effect of morphine, but did not antagonize the response to U50488H. RX8008M should prove a useful probe for the in-vivo characterization of the receptor selectivity of opioid drugs.

Opioid agonists selective for both μ and κ receptors produce antinociception in a variety of animal tests (Martin et al 1976; Tyers 1980). Although the comparison of agonist potencies in a variety of antinociceptive tests is of use in determining the μ/κ selectivity of opioid drugs (Tyers 1980) critical evaluation of receptor selectivity relies on the use of selective opioid antagonists.

16-Methylcyprenorphine (RX8008M) has been characterized in isolated tissue preparations as a pure opioid antagonist with high affinity for μ and δ opioid receptors and low affinity for κ receptors (Smith 1987). In the present study the effect of RX8008M on the antinociceptive action of the μ-selective agonist morphine and the κ-selective agonist U50488H has been investigated in the mouse abdominal constriction test.

Materials and methods

The procedures used to evaluate antinociceptive activity in the mouse abdominal constriction test have been described by Tyers (1980). Briefly, 30 min after subcutaneous administration of opioid agonists (dose volume 0·2 mL/20 g, dissolved in saline), mice (male, CRH, 18–22 g) were injected with acetylcholine, 3 mg kg^{-1} intraperitoneally, and the number of abdominal constrictions occurring in the first 4-min period thereafter recorded. The ED50 value was defined as the dose of test drug capable of reducing by 50% the number of abdominal constrictions occurring in placebo-treated mice. In studies investigating the effect of RX8008M, the antagonist was usually co-administered subcutaneously with agonist drugs, although in some experiments RX8008M was administered at varying times before and after the agonist. Individual tests were carried out in dose groups of six animals and data were accumulated from two or three individual tests carried out on different days. In all tests, animals and drugs were colour-coded so that the operators were unaware of the treatments the animals were receiving. Antinociceptive activity (ED50), 95% confidence limits and potency ratios, where applicable, were calculated by the method of Finney (1964).

Drugs used were as follows: acetylcholine hydrochloride (Sigma), morphine hydrochloride (McFarlan-Smith), U50488H (*trans*- (±) -3,4-dichloro-*N*-methyl-*N*-[2- (1-pyrollidinyl) cyclohexyl]benzene acetamide methane sulphonate [Upjohn]), 16-methylcyprenorphine (synthesized by Dr A. McElroy, Chemical Research, Glaxo).

Results

In the mouse abdominal constriction test, the μ-selective agonist morphine and the κ-selective agonist U50488H inhibited acetylcholine-induced abdominal constrictions with ED50 (95% confidence limits) values of 0·39 (0·29–0·55) and 0·34 (0·16–0·74) mg kg^{-1}, respectively. Subcutaneous co-administration of RX8008M (0·33, 1 and 3 mg kg^{-1} s.c.) produced a dose-dependent shift in the dose-response curve to morphine (dose ratios were: 8·6 (5·9–12·5), 15·7 (10·3–23·3) and 52·8 (37·0–76·9), respectively, n = 24 (see Fig. 1). Administration of RX8008M, 1 mg kg^{-1} s.c., 60 or 30 min before morphine produced only 2·2-fold (1·2–4·0) and 3·7-fold (2·1–6·7) shifts (n = 6), respectively, in the morphine dose-response curve. Administration of RX8008M (1 mg kg^{-1} s.c.) 15 min after morphine produced a 17·8-fold (11·6–27·8) shift (n = 12) in the morphine dose-response curve. Subcutaneous co-administration of RX8008M at doses of 10 and 30 mg kg^{-1} did not antagonize the response to U50488H, n = 12 (Fig. 2).

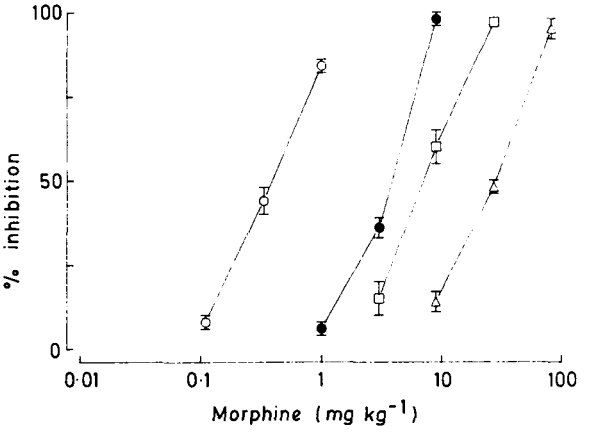

FIG. 1. Effect of co-administration (30 min pretreatment) of RX8008M on the antinociceptive action of morphine in the mouse abdominal constriction test. Morphine alone (○), morphine in the presence of RX8008M, 0·3 mg kg^{-1} s.c. (●), 1 mg kg^{-1} s.c. (□), 3 mg kg^{-1} s.c. (△). Values are means (± s.e.) with n = 24.

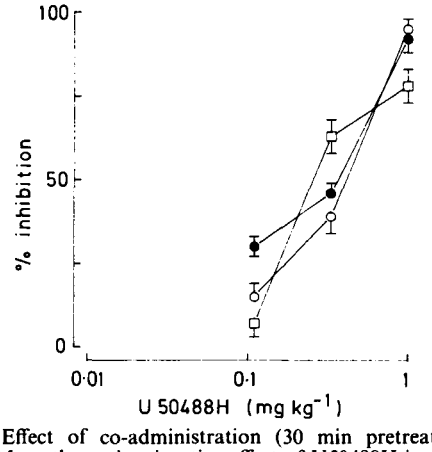

FIG. 2. Effect of co-administration (30 min pretreatment) of RX8008M on the antinociceptive effect of U50488H in the mouse abdominal constriction test. U50488H alone (○), U50488H in the presence of RX8008M, 10 mg kg^{-1} s.c. (●), 30 mg kg^{-1} s.c. (□). Values are means (± s.e.) with n = 12.

Correspondence to: P. J. Birch, Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DJ, UK.

Discussion

16-Methylcyprenorphine is a competitive opioid antagonist with Ke values at μ and κ receptors of 1·8 and 60 nM, respectively, and thus is one of the most selective competitive antagonists for distinguishing between μ and κ receptors reported to date (Smith 1987). In the present study, experiments have been performed to investigate the in-vivo selectivity of this antagonist.

16-Methylcyprenorphine produced a dose-dependent antagonism of the antinociceptive effect of μ-selective agonist morphine without antagonizing the response to the κ-selective agonist U50488H. The effect of RX8008M was short-lived as administration 60 or 90 min before testing produced only small shifts in the agonist dose-response curve. The results indicate that RX8008M maintains its antagonist selectivity in-vivo and should prove a useful probe for determination of the μ/κ selectivity of opioid drugs. It must be stated, however, that RX8008M has high affinity for the δ opiod receptor (Smith 1987) and would not distinguish between μ and δ receptor-mediated effects.

Recently, a cyclic somatostatin octapeptide (D-Phe-Cys-Tyr-D-Try-Lys-Thr-Pen-Thr NH_2) has been reported to be a selective μ opioid receptor antagonist in-vitro and in-vivo (Shook et al 1987 a, b). However, in-vivo studies with CTP require intracerebroventricular or intrathecal administration. Similarly, the irreversible opioid antagonist β-funaltrexamine can also be used to determine the μ/κ selectivity of opioid drugs (Hayes et al 1987). However, in-vivo studies require a 24 h pretreatment at relatively high dose levels and, due to the irreversible nature of the antagonism, interpretation of in-vivo data can be more difficult. Thus, RX8008M offers considerable advantages over CTP and β-funaltrexamine for determining the μ/κ selectivity of opioid drugs in view of its competitive nature of antagonism and its effectiveness after subcutaneous administration.

Obviously a κ-selective antagonist would be of equal importance in the characterization of opioid agonist selectivity. In this respect, Portoghese et al (1987) have recently reported the synthesis of two potent κ-selective antagonists, binaltorphimine and norbinaltorphimine. Studies are at present underway to investigate the in-vivo profile of these antagonists.

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