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Effect of β-funaltrexamine on opioid side-effects produced by morphine and U-50, 488H

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Pretreatment of rats with the irreversible μ-opioid receptor antagonist, β-funaltrexamine (β-FNA), 20–40 mg kg^{-1} s.c., produced a dose-related antagonism of the reduction in respiratory rate, gastrointestinal (GI) propulsion, rotarod reaction latencies and body temperature produced by morphine administration 24 h later, suggesting that these effects are mediated via μ-opioid receptors. The κ-receptor agonist, U-50,488H, was without effect on respiratory rate at the doses tested, but produced hypothermia, sedation and low maximum inhibition of GI propulsion. These effects of U-50, 488H were not blocked by β-FNA suggesting that they are mediated via κ-receptors.

There is now considerable evidence for the existence of at least three different opioid receptor subtypes, μ, κ and δ (Martin et al 1976, Lord et al 1977). Antinociceptive activity can be produced by activation of both μ and κ-receptors (Tyers 1980). However, there is considerable controversy as to which receptor subtypes are involved in producing undesirable opioid effects, such as constipation and respiratory depression.

From previous studies in this laboratory (Tyers 1980; Hayes & Tyers 1983), we have concluded that μ-receptors mediate both antinociception and side-effects like constipation, respiratory depression, mydriasis and hypothermia in the mouse. Furthermore, whereas κ-receptors also mediate antinociception, they only play a minor role in producing these undesirable effects. Porreca et al (1982), measuring agonist affinities in-vivo using buprenorphine as an 'irreversible' μ-antagonist, also concluded that morphine produces both analgesia and inhibition of gastrointestinal (GI) propulsion via the same receptor, presumably the μ-receptor. Florez & Pazos (1982), calculating apparent pA_2 values for naloxone for antagonizing morphine induced antinociception and respiratory depression, concluded that these two effects are also both mediated via the same receptor, the μ-receptor. However, McGilliard & Takemori (1978), using a similar experimental model, came to the opposite conclusion, i.e. that they are mediated via different receptors. Pasternak and his co-workers (Holaday et al 1983; Ling et al 1983) have suggested a subdivision of μ-receptors into high affinity μ_1 binding sites, responsible for opioid-induced analgesia, and low affinity μ_2 binding sites, responsible for respiratory and cardiovascular depression.

The purpose of the present experiments was to investigate further the receptors responsible for producing undesirable opioid effects, by studying the effect of the irreversible μ-receptor antagonist β-funaltrexamine (β-FNA), (Ward et al 1982a) on respiratory depression, hypothermia, sedation and inhibition of GI propulsion produced by a μ-agonist, morphine, and a κ-agonist, U-50,488H (VonVoigtlander et al 1983).

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Methods

Male PVG rats, 35–70 g, were used. The animals were injected subcutaneously, 24 h before testing, with either β-FNA or saline. Pilot studies had shown that 24 h was the optimum pretreatment time for β-FNA, later times producing no greater effect and earlier times being complicated by the agonist effects of β-FNA. On the day of test, agonist drugs were administered subcutaneously and, 30 min later, the following parameters were determined sequentially in each rat in the following order: respiratory rate, body temperature, rotarod reaction latency and inhibition of GI propulsion. Details of the measurement of these parameters are given in Hayes & Tyers (1983).

Individual tests were carried out using dose-groups of 6 rats. Data for calculation of potency ratios were accumulated from 2 individual tests carried out on different days, such that final dose-groups comprised 12 animals. Each dose-group was randomized between cages and rats and drug solutions were colour coded such that the operators were unaware of which treatment the animals were receiving.

Dose response curves were plotted from calculated mean and standard error values. Relative potencies were estimated according to the methods of Finney (1978), using a parallel line assay technique.

Drugs

All drugs were dissolved in saline and administered in a dose volume of 0·4 ml/100 g weight. Doses given in the text for salts of drugs refer to the weight of base. The following drugs were used: morphine hydrochloride (MacFarlan Smith); U-50,488H (*trans*-(±)-3,4 dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl] benzeneacetamide methanesulphonate) (Upjohn); β-funaltrexamine hydrochloride (β-FNA) (synthesized by Dr C. Meerholz and Dr A. McElroy, Chemistry Research Dept, Glaxo Group Research Ltd, Ware).

Results

The μ-agonist, morphine, 0·3–9 mg kg^{-1} s.c., produced dose-related decreases in rotarod reaction latencies,

respiration rate and GI propulsion in the rat (Fig. 1). Low dose-levels (0·3–1 mg kg^{-1}), produced a slight increase in body temperature, followed at higher dose-levels by hypothermia. Pretreatment of the rats 24 h previously with β-FNA, 20 and 40 mg kg^{-1} s.c., produced no direct effects on these parameters, but caused a dose-related antagonism of the effects of morphine (Fig. 1 and Table 1), the dose-response curves being shifted to the right in an apparently parallel fashion. For the measure of body temperature, this meant that doses of morphine that were hypothermic in control animals produced hyperthermia in rats pretreated with β-FNA.

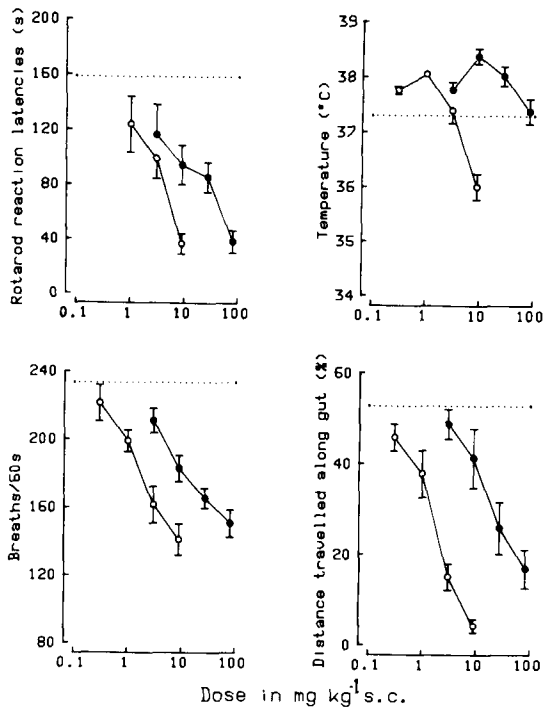

FIG. 1. Effect of β-FNA, 40 mg kg^{-1} s.c. given 24 h beforehand, on dose-response curves for morphine for decreasing rotarod reaction latencies, body temperature, respiratory rate and GI propulsion. The open circles represent saline pretreated rats and the closed circles β-FNA treated rats. The dotted lines represent mean values obtained for untreated controls. Each point is the mean ± standard error (n = 12).

The κ-receptor agonist, U-50,488H, produced dose-related decreases in rotarod reaction latencies, body temperature and gut propulsion, although the dose-response curve obtained for the latter effect was shallower than that obtained for morphine and a lower maximum effect was achieved (Fig. 2). U-50,488H did not produce any significant respiratory depression. Rats pretreated with β-FNA, 40 mg kg^{-1} s.c., did not show any significant changes in the dose-response curves obtained for these parameters (Fig. 2 and Table 1).

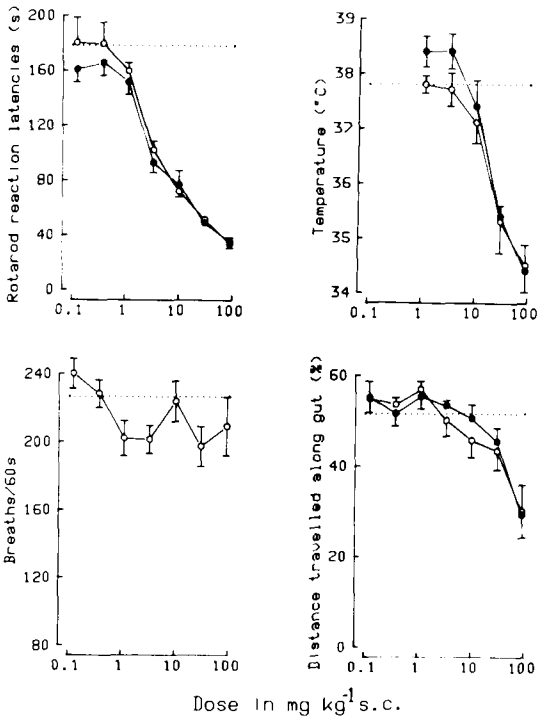

FIG. 2. Effect of β-FNA, 40 mg kg^{-1} s.c. given 24 h beforehand, on dose-response curves for U-50,488H for decreasing rotarod reaction latencies, body temperature, respiratory rate and GI propulsion. The open circles represent saline pretreated rats and the closed circles β-FNA treated rats. The dotted lines represent mean values obtained for untreated controls. Each point is the mean ± standard error (n = 12).

Table 1. Dose ratios for the antagonism by β-FNA of opioid side-effects produced by morphine and U-50, 488H.

Opioid side-effect	Dose of β-FNA (mg kg^{-1} s.c.)	Dose ratio (confidence/limits) Morphine	U-50, 488H
Sedation (rotarod)	20	4·1 (2·4–7·8)	—
	40	7·9 (4·3–13·3)	0·8 (0·6–1·3)
Respiratory depression	20	1·9 (0·7–4·3)	—
	40	7·9 (4·2–14·5)	
Inhibition of GI motility	20	8·0 (4·4–16·5)	—
	40	16·4 (10·0–28·1)	1·4 (0·6–3·8)
Hypothermia	20	—	—
	40	>5·1*	1·2 (0·8–1·9)

* After β-FNA treatment, a significant dose-related effect was not obtained.

Discussion

β-FNA has been shown to antagonize μ-opioid receptor-mediated effects, whilst having no effect on

κ-receptors, both in-vitro (Ward et al 1982a) and in-vivo (Ward et al 1982b). More recent studies have suggested that β-FNA also antagonizes δ-receptors in-vitro (Corbett et al 1985). However, as neither morphine nor U-50, 488H interact significantly with the δ-receptor (Magnan et al 1982; James & Goldstein 1984) this seems unlikely to complicate the interpretation of the results reported here. In this study, β-FNA produced a dose-related antagonism of morphine-induced depression of respiration rate, gastrointestinal transit, body temperature and rotarod reaction latencies. β-Funaltrexamine, at the same doses, also antagonizes morphine-induced antinociception in the rat (unpublished observations), which suggests that both the antinociceptive and the undesirable side-effect of morphine are mediated predominantly via the same receptor, presumably the μ-receptor.

Ward & Takemori (1983a) reported that β-FNA, 40 mg kg^{-1} s.c., produced a 10-fold shift of the morphine dose-response curve for inhibiting GI motility in the mouse. This value is comparable to the 16-fold shift obtained in these experiments in the rat. However, the antagonism of morphine-induced respiratory depression by β-FNA in the mouse was not clear cut, the result depending on the method of measurement used (Ward & Takemori 1983b). In this study, we have unequivocally shown antagonism of morphine-induced respiratory depression in the rat. The biphasic effect of morphine on body temperature in the rat, seen in these experiments, was also noted by Geller et al (1983). They suggested that the hyperthermia seen at low doses was μ-mediated and the hypothermia seen at higher doses was κ-mediated; since both components were antagonized by β-FNA in the present study, it seems likely that they are both mediated via μ-receptors. However, as U-50,488H-induced hypothermia was not antagonized by β-FNA, this suggests that hypothermia can also be mediated via κ-receptors.

U-50,488H, the selective κ-agonist (VonVoigtlander et al 1983), produced a slightly different profile of opioid side-effects to morphine. It was without significant effect on respiration rate in the rat, and although it did decrease GI propulsion, the maximum effect achieved was less than that of morphine. This effect of U-50,488H on GI motility was not antagonized by β-FNA, so it can be concluded that it is not μ-receptor, but presumably κ-receptor mediated. It seems that κ-receptors play a lesser role than μ-receptors in inhibiting gastrointestinal transit, possibly because the site of action of μ-agonists is thought to be both central and peripheral, whereas that of κ-agonists is only peripheral (Porreca et al 1983). The sedation and hypothermia produced by U-50,488H were also unaffected by β-FNA, again suggesting that these may be κ-mediated effects.

In conclusion, these studies with β-FNA suggest that opioid side-effects like sedation, hypothermia and inhibition of GI propulsion can be mediated by both μ and κ-opioid receptors. Respiratory depression appears to be largely μ-receptor mediated.

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