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Reversal by β-funaltrexamine of the antinociceptive effect of opioid agonists in the rat

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1 The effect of the irreversible opioid receptor antagonist, β-funaltrexamine (β-FNA), on antinociception produced by μ- and κ-receptor agonists was studied in the rat.

2 β-FNA, 20 to 80 mg kg^{-1}, s.c., given 24 h before testing, produced a dose-related antagonism of the effects of morphine in the paw pressure, hotplate and tail-flick tests. Following the 80 mg kg^{-1} dose, the degree of antagonism of morphine was stable for up to 48 h after dosing, but was reduced by 5 days and had disappeared by 8 days.

3 In the paw pressure test, β-FNA, 40 mg kg^{-1}, s.c., antagonized the effects of fentanyl, buprenorphine, tifluadom, ethylketocyclazocine and proxorphan; it was without effect against the highly selective κ-agonist, U-50,488.

4 In light of these results, the possible opioid receptor selectivities of both the agonists and β-FNA are reassessed.

Introduction

The existence of at least three distinct types of opioid receptor, μ, κ and δ, has been suggested from a variety of *in vitro* and *in vivo* experiments (Martin *et al.*, 1976; Lord *et al.*, 1977; Kosterlitz *et al.*, 1981). However, determination of the functional correlates of these receptor types has advanced slowly, largely because of the lack of selective opioid antagonists. Naloxone does not distinguish adequately between the different opioid receptor types (Sawynok *et al.*, 1979). Recently, it has been suggested that the non-equilibrium antagonist β-funaltrexamine (β-FNA) is selective for μ-receptors (Ward *et al.*, 1982a); although studies in the mouse and hamster isolated vas deferens have shown that β-FNA also possesses non-competitive antagonist actions at δ-receptors (Corbett *et al.*, 1985; Hayes *et al.*, 1985a).

Studies with selective opioid agonists have shown that antinociception can be mediated via both μ- and κ-receptors (Tyers, 1980; Upton *et al.*, 1983); the role of δ-receptors is more controversial (Ronai *et al.*, 1981; Chaillet *et al.*, 1984; Galligan *et al.*, 1984). Further evidence for a role of μ- and κ-receptors in antinociception has been obtained from antagonist studies. Thus, Ward & Takemori (1983) and Tyers (1983) obtained different pA_2 values for the antagonism by naloxone of the effects of μ- and κ-agonists in the mouse writhing test. Furthermore, Ward *et al.* (1982b), investigating the effects of β-FNA in the mouse, demonstrated antagonism of the antinociceptive effect of the μ-agonist morphine, but no antagonism of the κ-agonist nalorphine.

The purpose of the present study was to investigate further the opioid receptors involved in antinociception in the rat, by studying the interaction of β-FNA with a wider range of opioid agonists, including several μ- and κ-selective agents.

Methods

Male PVG rats, weighing 35–70 g, were used. The animals were injected subcutaneously, 1,2,5 or 8 days before testing, with either β-FNA or saline. On the day of test, agonist drugs were administered subcutaneously and 30 min later nociceptive thresholds were determined as described below. Individual tests were carried out using dose-groups of 6 rats. Data for calculation of dose-ratios were accumulated from 2 or 3 individual tests carried out on different days, such that final dose-groups comprised 12 or 18 animals. Each dose-group was randomized between cages and rats, and labels of drug solutions were colour coded such that the operators were unaware of which treatment the animals were receiving.

For plotting of dose-response curves, median and interquartile ranges were calculated. Relative potencies were estimated according to the methods of Finney (1978), by use of a parallel line assay technique.

Antinociceptive testing

Paw pressure test Nociceptive pressure thresholds were determined for the left hind paw with an 'Analgesymeter' (Ugo Basile, Milan). On obtaining the nociceptive response, which was a vocalization or paw withdrawal, the pressure being applied to the paw was released.

Hotplate test Reaction times of rats placed on a copper plate heated to 55 ± 0.2°C were determined. A 'front paw lick' was taken as the nociceptive response at which time the animal was rapidly removed from the hot plate. The maximum reaction time was taken as 60 s.

Tail flick test A beam of light was focused onto the rat's tail at a point midway along the dorsal surface and the latency for the animal to flick its tail out of the beam was measured automatically; 10 s was taken as the maximum reaction time.

Drugs

All drugs were dissolved in saline and administered in a dose volume of 0.4 ml 100 g^{-1} body weight. Doses given for salts refer to the parent compound. The following drugs were used: morphine hydrochloride (MacFarlan Smith); U-50,488 (*trans*-(±)-3,4 dichloro-*N*-methyl-*N*- [2- (1-pyrrolidinyl) cyclohexyl] benzeneacetamide methane sulphonate) (Upjohn); proxorphan tartrate (Bristol); ethylketocyclazocine methane sulphonate (Sterling-Winthrop); tifluadom hydrochloride (Sandoz); fentanyl citrate (Janssen); buprenorphine hydrochloride (Reckitt and Colman); β-funaltrexamine hydrochloride (β-FNA) (synthesized by Dr C. Meerholz and Dr A. McElroy, Chemical Research Dept., Glaxo Group Research Ltd., Ware).

Results

β-Funaltrexamine (β-FNA), 40 mg kg^{-1}, s.c., had no significant effect on nociceptive pressure thresholds either acutely (threshold at 30 min after dosing = 188 ± 9.6 g for saline pretreated rats and 218 ± 13.2 g for β-FNA pretreated rats; $P > 0.05$, $n = 10$), or chronically, at 1–8 days after dosing.

β-Funaltrexamine, 20–40 mg kg^{-1}, s.c., given 24 h before testing, produced progressive rightwards shifts of the morphine dose-response curves in the paw pressure, hotplate and tail-flick tests (Figure 1). A higher dose of β-FNA, 80 mg kg^{-1}, s.c., produced further shifts of the morphine dose-response curves in all three tests. In the rat paw pressure test the morphine dose-response curve was shifted in a parallel fashion, but in the hotplate and tail-flick tests, no antinociceptive effect was obtained with morphine at dose-levels up to 80 mg kg^{-1}, s.c. (Table 1). At 80 mg kg^{-1} β-FNA, approximately one third of the animals died within 1–2 h of dosing, possibly due to

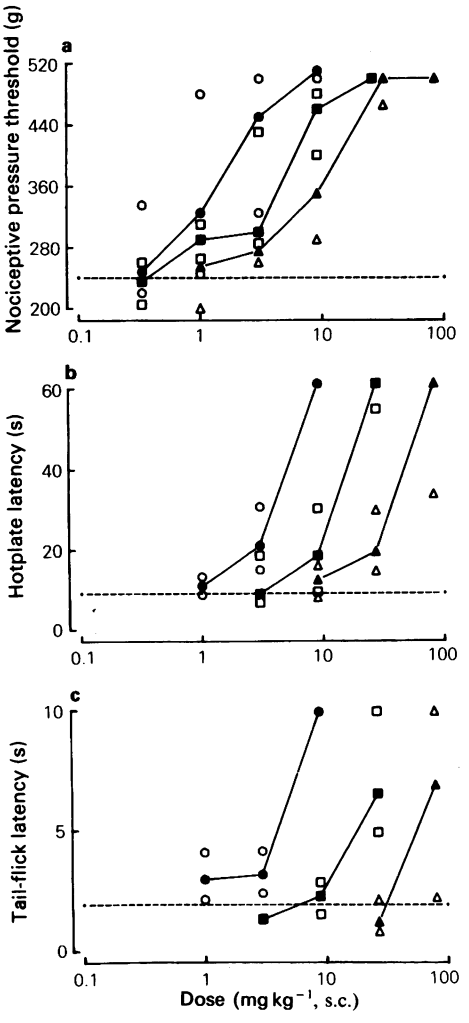

Figure 1 Effect of β-funaltrexamine (β-FNA), given 24 h before testing, on morphine-induced antinociception in the paw pressure (a), hotplate (b) and tail-flick (c) tests. Values are medians shown by the filled symbols, and interquartile ranges, shown by the open symbols ($n = 12$). The circles represent values for saline pretreated rats, the squares for rats pretreated with β-FNA 20 mg kg^{-1}, s.c., and the triangles for rats pretreated with β-FNA 40 mg kg^{-1}, s.c. The dotted lines represent values for controls.

Table 1 Antagonism of morphine-induced antinociception in the paw pressure, hotplate and tail-flick tests by β-funaltrexamine

Dose of β-FNA (mg kg^{-1}, s.c.)	*Antagonism of morphine Dose-ratio (Confidence limits) (n = 12)*		
	Paw pressure	*Hotplate*	*Tail-flick*
20	2.2(1.4–3.5)	3.5(2.3–5.3)	6.3(3.9–10.6)
40	4.7(3.0–7.5)	12.2(8.7–17.2)	19.5(14.3–29.3)
80	11.6(5.9–27.2)	>31	>27

the opioid agonist effects of the drug (Ward *et al.*, 1982b).

The degree of antagonism of morphine-induced antinociception by β-FNA, 80 mg kg^{-1} s.c., was similar at 24 and 48 h after dosing (Table 2), but was reduced by 5 days and was virtually non-existent by 8 days.

Based on these studies with morphine, a standard dose-level of β-FNA of 40 mg kg^{-1}, s.c., given 24 h before testing, was used to study its interaction with a number of other opioid drugs in the paw pressure test. The results are shown in Figure 2. In these studies, the dose-response curve for U-50,488 was not significantly shifted by β-FNA (dose-ratio = 1.7, confidence limits 1.0–2.7; $n = 18$). But, the antinociceptive effects of all the other opioid agonists tested, fentanyl, buprenorphine, tifluadom, ethylketocyclazocine (EKC) and proxorphan were significantly reduced by β-FNA. Parallel rightward shifts of the fentanyl and tifluadom dose-response curves were obtained (dose-ratio for fentanyl = 7.2, confidence limits 4.9–10.4, $n = 12$; dose-ratio for tifluadom = 4.0, confidence limits 2.6–6.0, $n = 12$). The EKC dose-response curve was shifted significantly to the right after β-FNA pretreatment but a dose-ratio could not be calculated as the dose-response curves were not parallel. The buprenorphine and proxorphan dose-response curves showed a decreased slope and depression of maximum effect.

Discussion

It has been suggested from *in vitro* studies that β-FNA irreversibly antagonizes μ-opioid receptors, whilst having no antagonist effect on κ-receptors (Ward *et al.*, 1982a). In the experiments described here, β-FNA prevented the antinociceptive effects of the μ-agonists morphine and fentanyl, confirming its μ-antagonist activity *in vivo*. Thus, after 24 h pretreatment, β-FNA produced a dose-related antagonism of the effects of morphine in the paw pressure, hotplate and tail-flick antinociceptive tests in the rat. The antagonism was still present at 5 days after dosing, confirming that the binding of β-FNA is irreversible. The size of the shifts obtained with β-FNA in the rat were comparable to those reported by other authors in the mouse (Ward *et al.*, 1982b; Hynes *et al.*, 1984). It is interesting to note that larger shifts of the morphine dose-response curve were obtained in the heat nociceptive tests as compared to the paw pressure test. The reason for this is not clear. The shifts obtained in all three tests were parallel for β-FNA doses up to 40 mg kg^{-1}, s.c., and so the dose-ratios should have been similar in all three tests. The fact that they were not, may suggest that the pressure and heat tests have different sites of action, with different accessibility to β-FNA. Despite the smaller dose-ratios obtained using the pressure nociceptive stimulus, this test was chosen for further interaction studies as heat tests are insensitive to certain types of opioid agonist (Harris & Rosenberg, 1967; Tyers, 1980).

It has been suggested that, although β-FNA lacks κ-antagonist properties, it produces an acute agonist effect via κ-receptors (Ward *et al.*, 1982a). Thus, β-FNA has short-lasting reversible agonist actions both *in vitro*, in guinea-pig ileum (Ward *et al.*, 1982a) and rabbit vas deferens (Hayes & Kelly, 1985), and *in vivo*, in the mouse writhing test (Ward *et al.*, 1982b), and these effects do appear to be mediated predominantly via κ-opioid receptors. However, unlike other κ-agon-

Table 2 Timecourse of the antagonism of morphine-induced antinociception by β-funaltrexamine 80 mg kg^{-1}, s.c.

Pretreatment time	*Antagonism of morphine Dose-ratio (Confidence limits) (n = 6)*	
	Paw pressure	*Hotplate*
24 h	11.6(5.9–27.2)	>31
48 h	13.7(8.6–22.5)	23.8(12.3–42.2)
5 days	3.4(2.6–4.4)	7.4(5.0–10.7)
8 days	1.7(1.1–2.6)	—

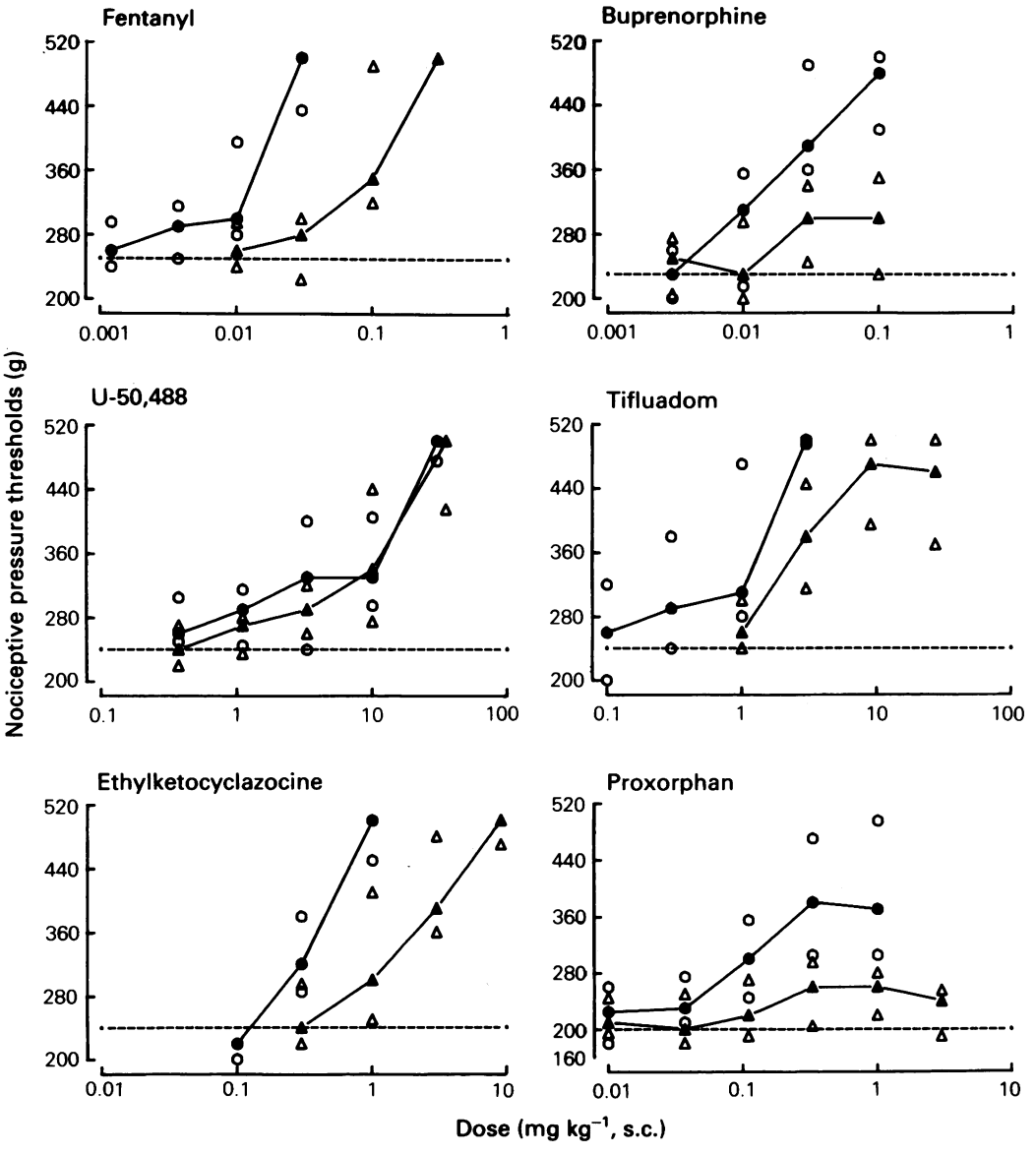

Figure 2 Effects of β-funaltrexamine (β-FNA), 40 mg kg^{-1}, s.c., given 24 h before testing, on the antinociceptive effects of some opioid agonists in the rat paw pressure test. Values are medians shown by the filled symbols, and interquartile ranges, shown by the open symbols (n = 12 or 18). The circles represent values for saline pretreated rats and the triangles for β-FNA pretreated rats. The dotted lines represent values for controls.

ists, β-FNA produces an acute antidiuretic rather than a diuretic effect in the rat (Zimmerman *et al.*, 1984), an action generally associated with μ-receptor activation (Huidobro, 1978). In the present experiments, β-FNA did not produce an antinociceptive effect in the rat either acutely or after 24 h, although it did show other acute actions indicative of opioid agonist activity e.g. hypothermia (unpublished observations).

The absence of κ-antagonist activity with β-FNA appeared to be confirmed by its lack of effect on the antinociception produced by the selective κ-agonist, U-50,488 (Gillan *et al.*, 1983; VonVoigtlander *et al.*,

1983). However, it did antagonize the effects of buprenorphine, EKC, tifluadom and proxorphan. Inhibition of the effect of buprenorphine was not unexpected as it has been suggested that this drug acts predominantly on μ-receptors (Martin *et al.*, 1976; Cowan *et al.*, 1977), although definitive *in vitro* studies on its opioid receptor selectivity have been difficult because of its high lipophilicity. Buprenorphine binds effectively to both μ- and κ-sites (Villiger, 1984), and in antinociceptive tests, buprenorphine is much more potent against pressure and chemical-induced nociception than against heat stimuli, a profile which is typical of both κ-agonists and partial μ-agonists (Tyers, 1980; Hayes *et al.*, 1985b). However, buprenorphine produces only antidiuresis in the water-loaded rat (Skingle *et al.*, 1985), an effect which is typically μ-receptor mediated, so it seems most likely that, in the rat, buprenorphine is also producing its antinociceptive activity via the μ-receptor. A depression of maximum was obtained with buprenorphine after β-FNA pretreatment, consistent with the view that this compound is a partial μ-agonist (Rance, 1979).

The reversal of the antinociceptive effects of EKC, tifluadom and proxorphan were unexpected in view of the fact that β-FNA does not block the agonist effects of these three compounds *in vitro* in guinea-pig ileum (Hayes *et al.*, 1985a). There are a number of potential explanations for this result. It is unlikely that β-FNA is blocking κ-receptors *in vivo* at the dose-levels used, as equivalent dose-levels of β-FNA did not inhibit the effects of U-50,488 in these experiments, nor do they block κ-mediated diuresis in the rat (Zimmerman *et al.*, 1984; Skingle *et al.*, 1985). Some κ-receptor antagonism occurs *in vitro* at high concentrations, although experiments in guinea-pig ileum suggest that its μ:κ selectivity is very high (Hayes *et al.*, 1985a). An alternative explanation is that EKC, tifluadom and proxorphan are producing their effects in the rat via μ-receptors. Indeed, rat brain is known to contain a much higher percentage of μ- than κ-receptors (Gillan & Kosterlitz, 1982), so it is a species where the μ-agonist effects of a compound are more likely to predominate. All three compounds are known to have affinity for the μ-receptor (Pircio & Buyniski, 1980; Gillan & Kosterlitz, 1982; Carroll *et al.*, 1984; James & Goldstein, 1984). Furthermore, EKC shows poor κ:μ agonist selectivity *in vivo* (Leander, 1983a); although tifluadom and proxorphan do show good agonist selectivity for the κ-receptor in a variety of systems (Leander, 1983b; Goldstein & James, 1984; Sheehan *et al.*, 1985). However, it is possible that these compounds are partial agonists at the μ-receptor, with sufficient efficacy to produce antinociception, but insufficient efficacy to produce agonist effects via the μ-receptor in other systems.

Recent studies have suggested that β-FNA antagonizes δ-receptors *in vitro* at concentrations similar to those that are effective in blocking μ-receptors (Corbett *et al.*, 1985; Hayes *et al.*, 1985a). However, it seems unlikely that interaction at δ-receptors could explain the anomalous results obtained here. There is controversy as to whether δ-receptors are involved in producing antinociception and, in any event, the affinity of both EKC and tifluadom is considerably lower at δ-sites than at either μ- or κ-sites (Garzon *et al.*, 1984; James & Goldstein, 1984).

In conclusion, we have shown that β-FNA antagonizes the antinociceptive effects of μ-receptor agonists in the rat, and also those of certain agonists that are generally considered to be κ-selective. Several potential explanations for these results are proposed, but further experiments are necessary to clarify the issue.

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