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Determination of the receptor selectivity of opioid agonists in the guinea-pig ileum and mouse vas deferens by use of β-funaltrexamine

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1 The irreversible inhibitor of μ-opioid receptor-mediated effects, β-funaltrexamine (β-FNA), was used to investigate the selectivity of various opioid agonists at μ-opioid receptors in the electrically stimulated guinea-pig ileum and mouse vas deferens preparations *in vitro*.

2 In the guinea-pig ileum, pretreatment with β-FNA (3×10^{-8}–3×10^{-6}M) produced a concentration-dependent antagonism of the inhibitory effect produced by the μ-opioid receptor agonist [D-Ala^2, MePhe^4, Gly(ol)^5]enkephalin (DAGO). High concentrations of β-FNA (3×10^{-6}–1×10^{-5}M) also antagonized the inhibitory effects of the κ-opioid agonist U50488.

3 Pretreatment of guinea-pig ileum with β-FNA at 1×10^{-6}M resulted in blockade of the effect of some opioid agonists. The compounds which showed the largest rightward shifts in their concentration-response curves, and hence the greatest μ/κ opioid receptor selectivity, were nalbuphine, [D-Ser^2, Leu^5]enkephalinyl-Thr^6(DSLET), morphine, DAGO and normophine. Responses to tifluadom, Mr 2034, ethylketocyclazocine, butorphanol, nalorphine, proxorphan and U50488 were not inhibited by β-FNA.

4 In the mouse vas deferens, pre-treatment with β-FNA (1×10^{-6}M) produced a similar shift in the dose-response curves for normophine as in the guinea-pig ileum. The concentration-response curves for the δ-receptor agonists [D-Ala^2, D-Leu^5] enkephalin (DADLE) and DSLET were, however, also shifted, indicating that β-FNA will also block δ-opioid receptors.

5 Since β-FNA does not block κ-opioid receptor-mediated effects, it can be used in the guinea-pig ileum preparation as a selective μ-receptor inhibitor. However, its lack of selectivity between μ- and δ-opioid receptors should be taken into account in many other isolated tissues and experiments *in vivo*.

Introduction

The alkylating analogue of naltrexone, β-funaltrexamine (β-FNA), was first synthesized by Portoghese *et al.* (1980). In isolated tissue preparations and *in vivo*, β-FNA initially causes a reversible opioid agonist effect, but on prolonged contact a selective irreversible blockade of responses mediated by μ-opioid receptors develops (Ward *et al.*, 1982a,b); the precise site and mechanism of action of β-FNA are uncertain. Ward *et al.* (1982a) suggested that β-FNA could thus be used to characterize the receptor selectivity of opioid agonists by measuring the reduction in potency that occurs after inhibition of the μ-receptor component of their action.

The guinea-pig ileum contains opioid receptors only of the μ- and κ-types (Ward & Takemori, 1976; Lord *et al.*, 1977) and so treatment with β-FNA should leave it responsive only to κ-opioid agonists. We have extended the findings of Ward *et al.* (1982a) to show that it is possible to produce a guinea-pig ileum preparation *in vitro* that may be devoid of functional μ-opioid receptors. Furthermore, by measuring the change in potency of a series of opioid agonists that occurs after β-FNA pretreatment, we have made an estimate of their relative μ/κ-receptor selectivity ratio.

The mouse vas deferens is a tissue that possesses δ-opioid receptors in addition to μ- and κ-opioid receptors (Lord *et al.*, 1977). Similar experiments in this tissue were carried out to study the effect of β-FNA on δ-receptors.

Methods

Guinea-pig ileum

The terminal ileum was removed from male Dunkin-

Hartley guinea-pigs (225–275 g), and the 15 cm section closest to the caecum discarded. After gently flushing out the contents, 1.5 cm sections were suspended in 5 ml organ baths containing a modified Krebs-Henseleit solution of the following composition (mM): NaCl 118, $NaHCO_3$ 29, glucose 11.1, KCl 4.7, $CaCl_2$ 2.6, KH_2PO_4 1.18 and $MgSO_4$ 4, gassed with 95% O_2 and 5% CO_2 and maintained at 37 ± 0.5°C. Each section of ileum was field-stimulated by two coaxial ring electrodes using rectangular pulses of 0.5 ms duration at 1V less than maximal voltage at a frequency of 0.1 Hz. Drugs were added to the bath in volumes of 10–50 μl. Non-cumulative concentration-response curves were constructed for an agonist and the reference drug, normorphine, and an IC_{50} concentration for 50% inhibition of twitch height was determined.

Tissues which were to be pretreated with β-FNA were incubated with it for 30 min. During this time the twitch height was depressed due to the agonist action of β-FNA. The tissues were then extensively washed with drug-free bathing medium for at least 60 min until the twitch height recovered and all non-covalently bound β-FNA was removed. Agonist concentration-response curves were then re-determined, and dose-ratios were calculated as (IC_{50} after β-FNA/IC_{50} before β-FNA).

For pA_2 determinations, four sections of ileum (normal or β-FNA-treated) from the same animal were incubated with naloxone for 30 min at a range of concentrations, each preparation being exposed to one concentration only. From the dose-ratio for agonist before and after β-FNA treatment, the pA_2 was calculated according to the method of Arunlakshana & Schild (1959). The statistical significance of the difference between the pA_2 values obtained in control and β-FNA-pretreated tissues was determined by use of Student's *t* test for unpaired samples.

Mouse vas deferens

Vasa deferentia were taken from male Glaxo-bred CRH mice (50–100 g) and suspended in 5 ml organ baths containing modified Krebs-Henseleit solution, but lacking magnesium sulphate. Each vas deferens was stimulated at 1V supramaximal voltage with trains of three 0.5 ms pulses 20 ms apart at a frequency of 0.1 Hz. Concentration-response curves were determined by cumulative dosing, and β-FNA was applied as described above for the guinea-pig ileum.

Sources of drugs

[D-Ala^2, D-Leu^5]enkephalin (DADLE), Peninsula; [D-Ala^2, MePhe^4, Gly(ol)^5]enkephalin (DAGO), Cambridge Research Biochemicals; bremazocine hydrochloride, Sandoz; butorphanol tartrate, Bristol Laboratories; clonidine hydrochloride, Boehringer Ingelheim; dihydrocodeine bitartate, Duncan Flockhart; ethylketocyclazocine methane sulphonate, Sterling Winthrop; [D-Ala^2, N-MePhe^4, Met-(0)-ol^5]enkephalin (FK 33824), Sandoz; fentanyl citrate, Janssen; ketocyclazocine, Sterling Winthrop; morphine hydrochloride, Macfarlan Smith; Mr 2034 ((−)-α-(1R,5R,9R)-5,9-dimethyl-2-(L-tetrahydrofuryl)-2′-hydroxy-6,7-benzomorphan), Boehringer Ingelheim; nalbuphine hydrochloride, Endo; nalorphine hydrobromide, Wellcome; naloxone hydrochloride, Sterling Winthrop; normorphine base, Wellcome; pentazocine hydrochloride, Sterling Winthrop; proxorphan tartrate, Bristol Laboratories; [D-Ser^2, Leu^5]enkephalinyl-Thr^6 (DSLET), Universal Biologicals; tifluadom hydrochloride, Sandoz; U50488 (*trans*-3,4-dichloro -N-methyl-N-[2-(1-pyrrollidinyl)cyclohexyl]benzeneacetamide methane sulphonate), Upjohn; Win 42610 (D-4-(cyclopropylmethyl) - 3,4,5,6 - tetrahydro - 1,5 - methano - 1,4- benzodiazocine-9-amine), Sterling Winthrop.

Results

As reported by Ward *et al.* (1982a), β-FNA produced a reversible inhibition of evoked contractions of the guinea-pig ileum. The IC_{50} for this opioid agonist effect was 9×10^{-9}M. More prolonged pretreatment of the ileum with β-FNA (3×10^{-8}–3×10^{-6}M, 30 min, followed by a 60 min period of frequent intermittent washout) produced concentration-dependent parallel rightward shifts of the concentration-response curve for the inhibitory effect of the μ-opioid receptor selective agonist, DAGO, ranging from 4.7 fold to 42 fold (Figure 1). The response to the selective κ-receptor agonist, U50488, was unaffected by pretreatment of the ileum with concentrations of β-FNA up to 1×10^{-6}M, but concentrations greater than 1×10^{-6}M did cause a parallel rightward shift of the dose-response curve (Figure 1). Such tissues took up to 2 h to recover their original twitch height. Control tissues not exposed to β-FNA showed only shifts of 2 fold or less in the IC_{50} to either agonist over the same period. Pretreatment with β-FNA (1×10^{-6}M or 1×10^{-5}M) also failed to shift the concentration-response curve to the α-adrenoceptor agonist, clonidine.

In both control and β-FNA-treated tissues, naloxone produced parallel shifts of the dose-response curve for DAGO. Table 1 shows the pA_2 values for antagonism of DAGO by naloxone in control segments of ileum and those treated with β-FNA (1×10^{-6}M). In control tissues, the pA_2 value of 8.58 was within the range for antagonism of μ-opioid receptors but after treatment of the preparations with 10^{-6}M β-FNA, the pA_2 fell to 7.78, a value consistent with an action of

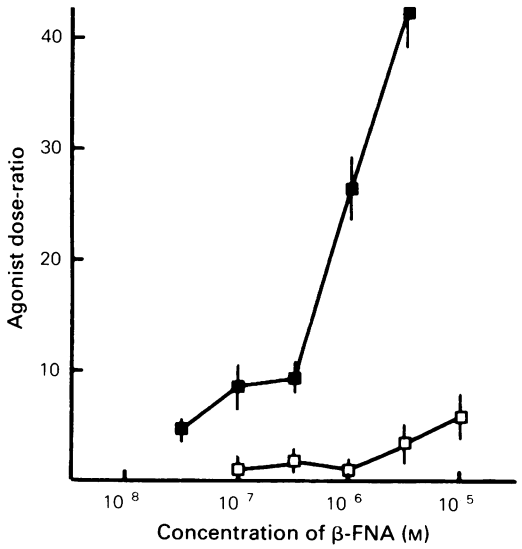

Figure 1 Concentration-dependence of the antagonism of the inhibitory effects of [D-Ala^2 MePhe^4, Gly(ol)^5]enkephalin (DAGO) and U50488 by β-funaltrexamine (β-FNA). The ordinate values are the dose-ratios (mean of 3–6 experiments, s.e.mean shown by vertical lines) for the μ-receptor selective agonist, DAGO (■) and the κ-receptor selective agonist, U50488 (□), after pretreatment of guinea-pig ileum with β-FNA at the concentrations shown, followed by 60 min intermittent washout with drug-free medium.

DAGO at κ-opioid receptors.

It can be seen from Figure 1 that the highest concentration of β-FNA which produced a shift of the DAGO concentration-response curve without affecting the response to U50488, was 1×10^{-6}M. This was therefore chosen as the standard treatment to investigate the effect of β-FNA on a range of opioid agonists. The dose-ratios for a series of opioid agonists which were obtained with this technique are shown in Table 2. For most agonists, the shifts were parallel and dose-ratios were calculated from IC_{50} concentrations.

Table 1 The pA_2 values for antagonism of [D-Ala^2, MePhe^4, Gly(ol)^5]enkephalin (DAGO) by naloxone in control and β-funaltrexamine (β-FNA)-pretreated guinea-pig ileum

Pretreatment	*Antagonism of DAGO*		
	pA_2	*Slope*	n
None	8.58 ± 0.12	0.92 ± 0.04	3
β-FNA 1×10^{-6}M	7.78 ± 0.12*	1.02 ± 0.08	5

Values are the means ± s.e.mean of *n* experiments. The asterisk indicates that the pA_2 is significantly lower than in untreated preparations ($P < 0.01$).

However, for the partial agonists DSLET, nalorphine, proxorphan and butorphanol, which did not usually cause a 50% inhibition of the twitch, the dose-ratios were calculated from parallel sections of the concentration-response curves. In the case of morphine and nalbuphine, the concentration-response curves after β-FNA often had significantly shallower slopes than in untreated tissues. The shift for nalbuphine tended to be smaller in tissues in which the maximum response to nalbuphine was low. The dose-ratios given in Table 2 are for the occasions when parallel shifts were obtained.

The effects of β-FNA were also investigated in the field-stimulated mouse vas deferens preparation. As in the guinea-pig ileum, β-FNA produced a concentration-dependent rightward shift in the concentration-response curve for normorphine while having very little effect on that for the κ-receptor selective agonist, ethylketocyclazocine. However, the concentration-response curves for the δ-selective agonists, DADLE and DSLET, were shifted significantly to the right. After treatment with β-FNA (1×10^{-6}M, 30 min) the

Table 2 Antagonist effects of β-funaltrexamine (β-FNA) against opioid agonists in the guinea-pig isolated ileum preparation

	Agonist dose-ratio	
Nalbuphine*	251 ± 97	(10)
DSLET*	50.6 ± 12.6	(4)
Morphine*	45.2 ± 5.4	(6)
DAGO	26.9 ± 2.3	(27)
Normorphine	17.6 ± 1.7	(14)
Fentanyl	14.6 ± 1.9	(3)
FK 33824	14.6 ± 1.5	(3)
Dihydrocodeine	13.6 ± 5.7	(4)
Pentazocine	3.8 ± 0.9	(5)
Tifluadom	2.5 ± 1.5	(3)
Mr 2034	2.2 ± 0.8	(3)
Ethylketocyclazocine	2.0 ± 0.3	(5)
Nalorphine*	1.8 ± 0.6	(6)
Win 42610	1.6 ± 0.4	(6)
Proxorphan*	1.5 ± 0.4	(4)
U50488	1.25 ± 0.33	(9)
Butorphanol*	1.14 ± 0.18	(6)
β-FNA	1.08 ± 0.33	(4)
Ketocyclazocine	0.90 ± 0.34	(5)

DSLET = [D-Ser^2, Leu^5]enkephalinyl-Thr^6; DAGO = [D-Ala^2, MePhe^4, Gly(ol)^5]enkephalin.
Tissues were treated with β-FNA, 1×10^{-6}M for 30 min, followed by a 60 min washout period. The dose-ratio was calculated as (IC_{50} after β-FNA/IC_{50} before β-FNA) except where indicated* (see text). Values are the means ± s.e.mean of (*n*) experiments. The dose-ratio given for β-FNA is for antagonism of its own agonist effect.

dose-ratios were as follows (mean ± s.e.mean of (*n*) preparations): normorphine 14.9 ± 3.0 (10), ethylketocyclazocine 1.2 ± 0.2 (12), DADLE 9.5 ± 1.6 (6), and DSLET 5.2 ± 1.1 (6). Experiments were performed in which the concentration of β-FNA and the time for which it was incubated were varied (Figure 2). In every combination tested, a large shift in the concentration-response curves for both normorphine and DADLE was seen, while the concentration-response curve for ethylketocyclazocine was almost unaffected.

Discussion

In the guinea-pig ileum, β-FNA was found to possess a reversible agonist action and an irreversible antagonist action, as reported by Ward *et al.* (1982a). The irreversible inhibitory activity was selective for μ-opioid receptor-mediated responses but high concentrations of β-FNA did shift the concentration-response curve for U50488 to the right, indicating that κ-opioid receptors may be blocked under such conditions. Under the incubation conditions used in these experiments, β-FNA appears to be about 100 times more selective for μ- than for κ-opioid receptors.

The means by which β-FNA blocks opioid receptor-mediated effects has not been determined. Portoghese & Takemori (1983) found that antagonists but not agonists would protect against β-FNA in the guinea-pig ileum and suggested that it acts not at the agonist binding site but at a receptor subunit which regulates the agonist site to which antagonists bind. However, the apparent lack of protection by agonists may have been at least partly a consequence of their much lower affinity for opioid receptors: the detrimental effect on the tissue precluded their use at concentrations likely to occupy an equivalent proportion of the receptors as the antagonists. Binding studies have produced conflicting results: McKnight *et al.* (1984) found that pretreatment of guinea-pig ileum myenteric plexus with β-FNA did not cause a reduction in the apparent number of binding sites for [^{3}H]-DAGO, whereas Rothman *et al.* (1983) reported large decreases in numbers of binding sites in rat brain membranes.

In the present study, the shifts in the concentration-response curves caused by β-FNA pretreatment in the guinea-pig ileum preparation were, with few exceptions, parallel. In a tissue containing a single population of receptors, the expected effect of β-FNA would be to cause a flattening of the concentration-response curve after exhaustion of the receptor reserve, since irreversible blockade of active receptors results in a reduction of the maximum agonist effect achievable (Furchgott, 1966). In the guinea-pig ileum, where opioids can act on two populations of receptors of which only one, the μ-receptor, is blocked by β-FNA

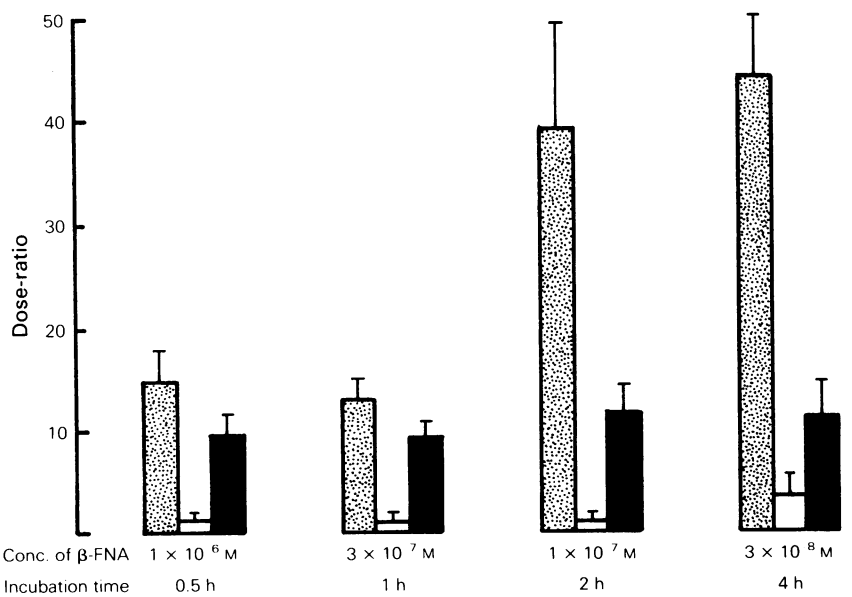

Figure 2 Irreversible antagonism by β-funaltrexamine (β-FNA) of μ-, κ- and δ-opioid receptor agonists in the mouse vas deferens. Columns represent the dose-ratio obtained for antagonism of normorphine (μ) (stippled column), ethylketocyclazocine (κ) (open column) and [D-Ala^2, MePhe^4, Gly(ol)^5]enkephalin (δ) (solid column) after prior incubation with β-FNA under the conditions shown. Values are the means $n = 5-12$; vertical lines show s.e.means.

pretreatment, a parallel shift of the concentration-response curve may indicate that the drugs exert their post-β-FNA agonist effect through the other opioid receptor, the κ-receptor. The change in the pA_2 value for antagonism of DAGO by naloxone after β-FNA pretreatment provides evidence to support this interpretation. Typically, naloxone pA_2 values for antagonism of μ-receptor agonists range from 8.5–9, while those for κ-receptor agonists range from 7.5–8.1 (e.g. Hutchinson *et al.*, 1975, Ward & Takemori, 1976, Hayes & Kelly, 1985 and our own unpublished observations). After pretreatment of the guinea-pig ileum with β-FNA (1×10^{-6}M, 30 min), the pA_2 for naloxone antagonism of DAGO thus changed from a value typical of its antagonist potency at μ-opioid receptors (8.58) to a value typical of its potency at κ-opioid receptors (7.78), suggesting that after such treatment most of the agonist action of DAGO was mediated through κ-opioid receptors. The further shift in the DAGO dose-response curve by pretreatment with β-FNA concentrations greater than 1×10^{-6}M is probably due to antagonism of its κ-opioid agonist component. Although β-FNA (1×10^{-6}M, 30 min) appeared to abolish the μ-receptor-mediated component of the response to DAGO, it should be noted, however, that it is not possible to be certain that there are no remaining μ-opioid receptors. The affinity of naloxone is only about ten times greater for μ- than κ-opioid receptors and so the naloxone pA_2 value for an agonist with only marginal selectivity for μ-over κ-opioid receptors is likely to be within the κ range.

The selective blockade of μ-opioid agonist action produced by β-FNA pretreatment in the guinea-pig ileum was used to characterize the μ-/κ-receptor selectivity of a range of drugs. Concentration-response curves to DSLET, DAGO, normorphine, fentanyl, FK33824, dihydrocodeine and pentazocine were shifted to the right indicating that they are μ-selective opioid agonists in this tissue. This is supported by evidence from tissue preparations *in vitro* and from binding studies (Lord *et al.*, 1977; Gacel *et al.*, 1980; Gillan *et al.*, 1980; Leslie *et al.*, 1980). However, the small shift for pentazocine may indicate that although it is μ-receptor selective, it also has appreciable activity at κ-opioid receptors. All of the compounds that produced dose-ratios of 2.5 or less have previously been characterized as κ-receptor selective agonists (Martin *et al.*, 1976; Kosterlitz & Leslie, 1978; Merz & Stockhaus, 1979; Römer *et al.*, 1982; Vonvoigtlander *et al.*, 1983; Burkard, 1984), but binding studies show that some have comparable affinities for both μ- and κ-sites (e.g. Johnson & Pasternak, 1983, for Mr 2034; Kosterlitz *et al.*, 1981, for ethylketocyclazocine; Maurer, 1982 for bremazocine). When agonists show a low dose-ratio after β-FNA, it is reasonable to conclude that they are not μ-receptor selective: however, it is impossible to distinguish between agonists that are κ-selective and those that are non-selective.

In the mouse vas deferens, as in the guinea-pig ileum, β-FNA blocked μ-opioid receptors whilst having little effect on κ-opioid receptors. However, the effects of the enkephalin analogues DADLE and DSLET were also antagonized in this tissue. It is likely from the concentration at which they were active, from the antagonism by the selective δ-receptor antagonist, ICI 154129, and from previous work with these compounds (Kosterlitz *et al.*, 1980; Gacel *et al.*, 1980) that they were acting through δ-opioid receptors.

Thus β-FNA is able to block δ-receptors in addition to μ-receptors. Since the antagonism remained after extensive washing, it is presumably also irreversible. Furthermore, the concentration range at which β-FNA blocked δ-receptor-mediated effects was the same as that which was effective against μ-receptor mediated ones: none of the varying incubation times and concentrations that were tested showed any dissociation of the blockade of μ- and δ-effects. Thus we find that, contrary to the work of Ward *et al.* (1982a), β-FNA shows little or no selectivity between μ- and δ-opioid receptors, and this should be taken into account when carrying out studies with β-FNA *in vitro* and *in vivo*.

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References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BURKARD, W.P. (1984). [^{3}H]-tifluadom binding in guinea-pig brain membranes. *Eur. J. Pharmac.*, **97**, 337–338.

FURCHGOTT, R.F. (1966). The use of β-haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. *Adv. Drug Res.*, **3**, 21–55.

GACEL, G., FOURNIE-ZALUSKI, M.C. & ROQUES, B.P. (1980). D-Tyr-Ser-Gly-Phe-Leu-Thr, a highly preferential ligand for δ-opiate receptors. *FEBS Letters*, **118**, 245–247.

GILLAN, M.G.C., KOSTERLITZ, H.W. & PATERSON, S.J. (1980). Comparison of the binding characteristics of tritiated opiates and opioid peptides. *Br. J. Pharmac.*, **70**, 481–490.

HAYES, A. & KELLY, A. (1985). Profile of activity of κ receptor agonists in the rabbit vas deferens. *Eur. J. Pharmac.*, **110**, 317–322.

HUTCHINSON, M., KOSTERLITZ, H.W., LESLIE, F.M., WATERFIELD, A.A. & TERENIUS, L. (1975). Assessment in the guinea-pig ileum and mouse vas deferens of benzomorphans which have strong antinociceptive activity but do not substitute for morphine in the dependent monkey. *Br. J. Pharmac.*, **55,** 541–546.

JOHNSON, N. & PASTERNAK, G.W. (1983). The binding to rat brain homogenates of Mr2034, a universal opiate. *Life Sci.*, **33,** 985–991.

KOSTERLITZ, H.W. & LESLIE, F.M. (1978). Comparison of the receptor binding characteristics of opiate agonists interacting with μ- or κ-receptors. *Br. J. Pharmac.*, **64,** 607–614.

KOSTERLITZ, H.W., LORD, J.A.H., PATERSON, S.J. & WATERFIELD, A.A. (1980). Effects of changes in the structure of enkephalins and of narcotic analgesic drugs on their interactions with μ- and δ-receptors. *Br. J. Pharmac.*, **68,** 333–342.

KOSTERLITZ, H.W., PATERSON, S.J. & ROBSON, L.E. (1981). Characterisation of the κ-subtype of the opiate receptor in the guinea-pig brain. *Br. J. Pharmac.*, **73,** 939–949.

LESLIE, F.M., CHAVKIN, C. & COX, B.M. (1980). Opioid binding properties of brain and peripheral tissues: evidence for heterogeneity in opioid ligand binding sites. *J. Pharmac. exp. Ther.*, **214,** 395–402.

LORD, J.A.H., WATERFIELD, A.A., HUGHES, J. & KOSTERLITZ, H.W. (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature*, **267,** 495–499.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R. & GILBERT, P.E.(1976). The effects of morphine- and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. *J. Pharmac. exp. Ther.*, **197,** 517–532.

MAURER, R. (1982). Multiplicity of opiate receptors in different species. *Neurosci. Lett.*, **30,** 303–307.

McKNIGHT, A.T., PATERSON, S.J., CORBETT, A.D. & KOSTERLITZ, H.W. (1984). Acute and persistent effects of β-funaltrexamine on the binding and agonist potencies of opioids in the myenteric plexus of the guinea-pig ileum. *Neuropeptides*, **5,** 169–172.

MERZ, H. & STOCKHAUS, K. (1979). N-[(Tetrahydrofuryl)alkyl] and N-(alkoxyalkyl) derivatives of (−)-normetazocine, compounds with differentiated opioid action profiles. *J. med. Chem.*, **22,** 1475–1483.

PORTOGHESE, P.S., LARSON, D.L., SAYRE, L.M. & FRIES, D.S. (1980). A novel opioid receptor site directed alkylating agent with irreversible narcotic antagonistic and reversible agonistic activities. *J. med. Chem.*, **23,** 233–234.

PORTOGHESE, P.S. & TAKEMORI, A.E. (1983). Different receptor sites mediate opioid agonism and antagonism. *J. med. Chem.*, **26,** 1343–1348.

RÖMER, D., BUSCHER, H.H., HILL, R.C., MAURER, R., PETCHER, T.J., ZEUGNER, H., BENSON, W., FINNER, E., MILKOWSKI, W. & THIES, P.W. (1982). An opioid benzodiazepine. *Nature*, **298,** 759–760.

ROTHMAN, R.B., BOWEN, W.D., SCHUMACHER, U.K. & PERT, C.B. (1983). Effect of β-FNA on opiate receptor binding: preliminary evidence for two types of μ-receptors. *Eur. J. Pharmac.*, **95,** 147–148.

VONVOIGTLANDER, P.F., LAHTI, R.A. & LUDENS, J.H. (1983). U-50488: a selective and structurally novel non-mu (kappa) opioid agonist. *J. Pharmac. exp. Ther.*, **224,** 7–12.

WARD, S.J., PORTOGHESE, P.S. & TAKEMORI, A.E. (1982a). Improved assays for the assessment of κ and δ-properties of opioid ligands. *Eur. J. Pharmac.*, **85,** 163–170.

WARD, S.J., PORTOGHESE, P.S. & TAKEMORI, A.E. (1982b). Pharmacological characterisation *in vivo* of the novel opiate, β-funaltrexamine. *J. Pharmac. exp. Ther.*, **220,** 494–498.

WARD, A.W. & TAKEMORI, A.E. (1976). Studies on the narcotic receptor in the guinea-pig ileum. *J. Pharmac. exp. Ther.*, **199,** 117–123.

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