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Antinociceptive effects of the novel opioid peptide BW443C compared with classical opiates; peripheral versus central actions

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1 To investigate peripherally mediated antinociceptive effects of opioids, the activity of a novel polar enkephalin analogue H-Tyr-D-Arg-Gly-Phe (4-NO_2)-Pro-NH_2 (BW443C) has been compared with those of classical tertiary opiates against different nociceptive stimuli in the mouse.

2 In chemically-induced writhing models BW443C, administered subcutaneously, demonstrated dose-related antinociceptive effects less potent than morphine and of a similar order to pethidine and D-propoxyphene. In assays using heat as the noxious stimulus BW443C was markedly less potent than any of the opiates tested.

3 In heat-induced assays, but not in chemically-induced writhing assays, BW443C demonstrated a 'U'-shaped dose-time response relationship. Morphine, pethidine and D-propoxyphene showed simple, approximately linear, dose-time effects in all assays.

4 When given subcutaneously, the inhibitory effects of BW443C and morphine in chemically-induced writhing were antagonized by naloxone given intraperitoneally. The inhibitory effects on writhing of BW443C, but not those of morphine, were also antagonized by prior intraperitoneal administration of the quaternary opioid antagonist N-methyl nalorphine. When this antagonist was administered intracerebroventricularly, the antinociceptive effects in writhing of both BW443C and morphine were antagonized.

5 It is concluded that BW443C, being only poorly able to cross the blood brain barrier, demonstrates peripherally mediated opioid antinociceptive effects in chemically-induced writhing models. In heat-induced models, that detect centrally acting opioids, BW443C is effective only at high doses and at time intervals after dosing sufficient to allow slow penetration of drug into the CNS. It is suggested that the peripheral antinociceptive actions of BW443C are mediated by inhibition of sensory neurones.

Introduction

The analgesic actions of classical opiates such as morphine are accepted generally to be mediated centrally through an action at the supraspinal or spinal level. Antinociceptive effects, however, have been demonstrated to result also from local administration of opiates in the periphery, for example in mouse writhing (Bentley *et al.*, 1981) and rat paw models of acute inflammation (Ferreira & Nakamura, 1979; Ferreira *et al.*, 1981; Rios & Jacob, 1982; 1983). These antinociceptive effects have been attributed to opioid-induced actions mediated peripherally. One of the pharmacological probes which has been employed to discriminate peripheral and central actions is the use of quaternary salts which, bearing a permanent positive charge, show a restricted penetration of the blood brain barrier. Utilising such compounds derived from classical opiates, peripherally mediated antinociceptive effects of N-methyl morphine have been demonstrated (Smith *et al.*, 1982; 1985; Lorenzetti & Ferreira, 1982). Of equal, if not greater, importance in studies of this kind is the use of reliable peripherally selective quaternary opioid antagonists (Brown & Goldberg, 1985). A number of studies with quaternary opioid antagonists have been performed; for example, to antagonize peripherally-mediated effects of opiates on the gastrointestinal tract without antagonizing centrally-mediated antinociceptive effects (Bianchi *et*

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al., 1982; Russell et al., 1982) and to antagonize the antinociceptive effects of N-methyl morphine or of locally administered morphine (Smith et al., 1982; Lorenzetti & Ferreira, 1982; Ferreira et al., 1984).

We have sought to extend to peptides, studies on the putative peripheral action of opioids in antinociception by the introduction of the basic amino acid D-arginine into an enkephalin analogue. H-Tyr-D-Met-Gly-Phe-Pro-NH_2 (Bajusz et al., 1977; Szekely et al., 1977) and its Phe^4 (4-NO_2) analogue (Smith & Wilkinson, 1982) have been shown to be potent antinociceptive opioid peptides. By the introduction of D-Arg at position 2 in this latter molecule, a position known to tolerate a considerable degree of structural variation, a highly polar enkephalin analogue is obtained. We have shown recently that enkephalin analogues containing polar amino acid residues such as $D\text{-}Arg^2$ may retain a high degree of opioid activity (Hardy et al., 1987).

The present study describes the antinociceptive profile of H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2 (BW443C) in comparison with those of the classical centrally acting opioids morphine, D-propoxyphene and pethidine. The relative effects of the quaternary opioid antagonist N-methyl nalorphine, administered intraperitoneally and intracerebroventricularly, against BW443C and morphine in irritant-induced writhing have also been examined. Preliminary accounts of antinociceptive effects of BW443C have been published by Follenfant et al. (1987) and Lorenzetti & Ferreira (1987).

Methods

Writhing models in the mouse

Groups of at least 5 female Charles River mice of the CD1 strain or Tuck TFW strain (20–30g) were injected intraperitoneally with phenyl-*p*-benzoquinone (PBQ) 2.5 mg kg^{-1} or with 0.6% v/v acetic acid in dose volumes of 10 and 25 ml kg^{-1}, respectively. The irritants induced a series of abdominal contractions and/or hindlimb extensions (writhes) which were counted over 2.5–5 min periods commencing 10–20 min after PBQ or acetic acid injection. In both writhing models vehicle or drug was administered at a dose volume of 10 ml kg^{-1}. Antinociceptive activity was assessed in terms of an ED_{50} and determined by linear regression analysis. The ED_{50} was defined as that dose of drug which reduced by half the number of writhes obtained in PBQ challenged mice after vehicle administration. In antagonist studies, naloxone, N-methyl nalorphine or vehicle was injected intraperitoneally (10 ml kg^{-1}) 20 min before, or intracerebroventricularly (i.c.v.; 10 µl per mouse) 5 min before subcutaneous administration of the opioid agonists, which were given 30 and 10 min before the writhing agent, respectively.

Hotplate assay in the mouse

The method used was based on that of Woolfe & Macdonald (1944). Male Hacking and Churchill (CFLP strain; 20–35g) mice were used in groups of five. Mice were placed on the surface of the copper base of a perspex box partially immersed in a water bath at 55°C. Each mouse was observed for signs of discomfort such as licking or paw shaking for a maximum period of 30 s. Vehicle or drug was administered subcutaneously at a dose volume of 10 ml kg^{-1}.

The ED_{50} was defined as that dose of drug which induced a two fold increase in the reaction time on the hotplate. The ED_{50} was determined from quantal data by parallel line probit analysis.

Tailflick assay in the rat

The method used was based on that of Green et al. (1951). Female Wistar rats (100–200 g) were accustomed to being placed individually in a ventilated, restraining perspex tube. Rats were then exposed to radiant heat from a light source focussed on a small blackened section of the tail 1 cm from its tip. The reaction time for the rat to flick its tail out of the beam of light was determined within a 'cut off' time of 10 s. Drug or vehicle were injected subcutaneously in a dose volume of 5 ml kg^{-1}. ED_{50} determinations were carried out by parallel line probit analysis to calculate the dose of drug which doubled the reaction time of the tailflick.

Gastrointestinal propulsion in the mouse

The method used was based on that of Green (1959). Groups of at least 5 male Hacking and Churchill mice (CFLP strain; 20–35 g) were given a charcoal meal consisting of 1 part, by volume, powdered charcoal, 2 parts plain white flour and 6 parts water. Each mouse received 0.1 ml of the meal by intragastric tube. Fifteen min later the mice were killed and the distance the charcoal meal had travelled along the small intestine determined and expressed as a percentage of the total intestine length. Drugs were administered subcutaneously (10 ml kg^{-1}) 30 min before the meal. Potency was expressed as an ED_{50}, defined as the dose of drug the reduced the propulsion of the charcoal meal by 50% of that in saline-treated control mice, and derived by linear regression analysis.

In all assays the observer was blind to the drug treatment given. All drugs were dissolved in 0.85% w/v sodium chloride solution (saline) and doses are expressed in terms of the free base.

Materials

Drugs used: morphine hydrochloride (MacFarlan Smith), D-propoxyphene hydrochloride (Lilly), pethidine hydrochloride (Roche) and naloxone hydrochloride (SALARS). N-methyl nalorphine (Dr S. Wilkinson) and BW443C were synthesized in the Department of Medicinal Chemistry, Wellcome Research Laboratories.

Results

Antinociceptive effects

BW443C reduced in a dose-dependent manner the number of writhes elicited by intraperitoneal injections of two chemical irritants in mice (CD1 strain). As shown in Table 1, BW443C was approximately equipotent as an antinociceptive agent against PBQ- and acetic acid-induced writhing (ED_{50}s of 4.9 and 5.7 mg kg^{-1}, respectively) and, in these models, was of the same order of potency as D-propoxyphene (ED_{50}s of 4.2 and 9.8 mg kg^{-1}) and pethidine (ED_{50}s of 3.4 and 9.5 mg kg^{-1}). Morphine was a more potent antinociceptive agent against the irritants used (ED_{50}s of 0.4 and 0.5 mg kg^{-1}). BW443C and morphine were also investigated in acetic acid-induced writhing in a second strain of mouse (TFW). BW443C showed an increased potency in this strain (ED_{50} = 1.2 mg kg^{-1}: 95% confidence limits 0.6–2.0 mg kg^{-1}), whereas the potency of morphine was similar in the 2 strains of mouse (ED_{50} TFW = 0.8 mg kg^{-1}: 95% confidence limits 0.6–1.1 mg kg^{-1}). However, in the assays using heat as the noxious stimulus, BW443C was markedly less potent than the opiates tested. As shown in Table 1, compared with PBQ-induced writhing, BW443C was approximately 11 times less potent in the tailflick assay and 7 times less potent in the hotplate test. In contrast, D-propoxyphene for example was approximately 2 and 6 times less potent in tailflick and hotplate assays respectively compared with PBQ-induced writhing. Further differences in the antinociceptive activity of BW443C compared with that of the opiates was observed in the time course of effects in the chemical- and heat-induced assays.

Comparison of time courses in antinociceptive assay

As shown in Table 1, 30 min after subcutaneous administration, the order of potency for D-propoxyphene and pethidine in the antinociceptive assays was PBQ writhing > tailflick > acetic acid writhing > hotplate. Figure 1 shows the time course of antinociceptive activity (ED_{50}) for BW443C, D-propoxyphene and pethidine in these two writhing and two heat assays. It was observed, in all 4 models, that D-propoxyphene and pethidine demonstrated approximately linear correlations of antinociceptive activity with time, i.e. with increasing time after dosing, increasing doses of the opiates were required to produce equiactive effects.

In comparison the potency of BW443C in the four assays 30 min after subcutaneous administration was PBQ writhing = acetic acid writhing >> hotplate > tailflick (see Table 1 for details). With respect to the time course of antinociceptive activity of BW443C, whereas in PBQ- and acetic acid-induced writhing the relationship between activity and time was approximately linear, that relationship in the hotplate and tailflick assays was markedly 'U'-shaped (Figure 1). The potency differences of BW443C in the writhing assays compared with the heat assays, therefore, were most evident at short intervals after administration.

Antagonism by naloxone and N-methyl nalorphine

The antagonist effects of naloxone and N-methyl

Table 1 Antinociceptive potencies of opioids against chemical- and heat-induced models of nociception

	Antinociceptive activity ED_{50} (mg kg^{-1} s.c.)			
	Writhing assays		*Heat assays*	
Agonist	*PBQ*	*Acetic acid*	*Tailflick*	*Hotplate*
BW443C	4.9	5.7	56	35
	(3.7–6.2)	(4.9– 7.0)	(16–488)	(19–64)
Morphine	0.4	0.5	1.0	1.9
	(0.2–1.0)	(0.3– 0.6)	(0.5– 2.2)	(0.6–4.9)
D-Propoxyphene	4.2	9.8	9.5	26
	(3.0–5.4)	(8.2–12.0)	(5.5–16.0)	(16–40)
Pethidine	3.4	9.5	7.4	35
	(2.6–4.2)	(6.0–14.0)	(4.0–13)	(24–51)

Figures in parentheses are 95% confidence limits. The opioids were administered 30 min before the application of the noxious stimulus. At least 5 animals per dose group and a minimum of 4 doses were used for each ED_{50} determination. PBQ = phenyl-*p*-benzoquinone.

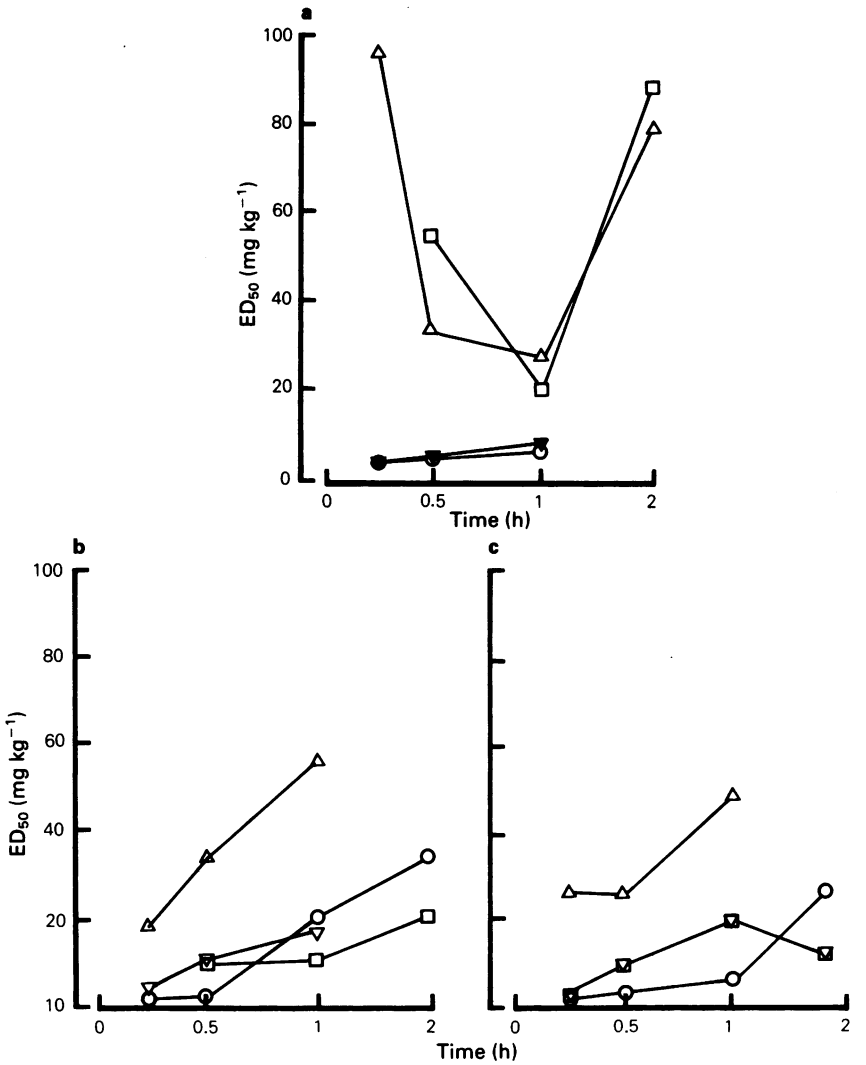

Figure 1 The effects of time (hours after dosing) on potencies (ED_{50}, mg kg^{-1}) of (a) BW443C, (b) pethidine and (c) D-propoxyphene in phenyl-p-benzoquinone-induced writhing (○), acetic acid-induced writhing (▽), hotplate test (△) in mice and tailflick test (□) in the rat.

nalorphine were investigated, after their intraperitoneal and intracerebroventricular administration, on acetic acid- and PBQ-induced writhing in mice (TFW and CDI strains, respectively).

The antinociceptive effects of BW443C in acetic acid-induced writhing were antagonised by prior intraperitoneal administration of the peripheral opioid antagonist N-methyl nalorphine, 7.5 and 15 mg kg^{-1} (Figure 2) and dose-ratios are shown in Table 2. The antinociceptive effects of morphine in this model, however, were not antagonized by N-methyl nalorphine (11.5 mg kg^{-1}, Figure 2). Naloxone (3.75 and 7.5 mg kg^{-1} i.p.) antagonized significantly the antinociceptive effects of both BW443C and morphine (Table 2).

When administered intracerebroventricularly, naloxone (0.3 μg per mouse) reduced the number of writhes induced by PBQ (8.4 ± 0.5 to 5.6 ± 0.5). The antinociceptive effects of subcutaneous BW443C and morphine were therefore expressed in terms of percentage inhibition in this model, and as shown in Figure 3, naloxone (0.3 μg per mouse i.c.v.) antagonized sig-

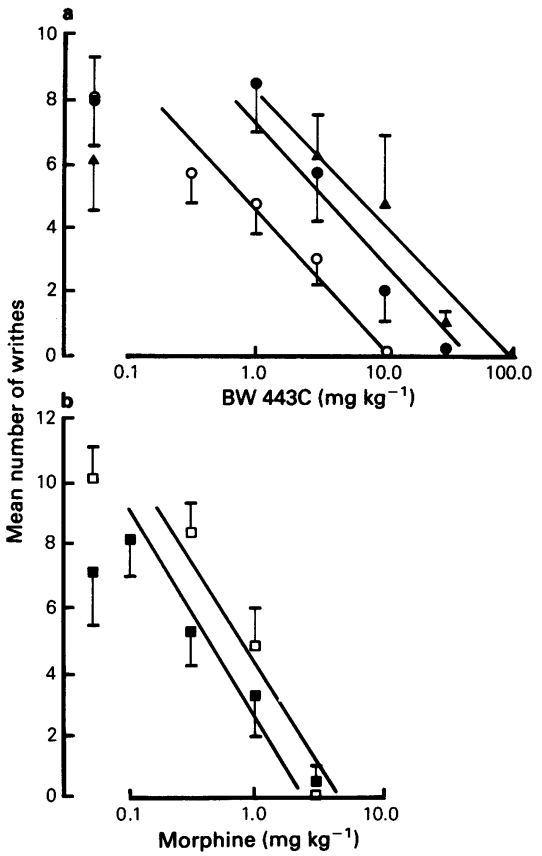

Figure 2 Mean number of writhes induced by acetic acid in mice in the presence of (a) BW443C (s.c.) and (b) morphine (s.c.) in the absence (○, □) or presence of N-methyl nalorphine (i.p.) 7.5 mg kg^{-1} (●), 11.5 mg kg^{-1} (■) and 15 mg kg^{-1} (▲). The vertical lines represent the s.e.mean; each point is the mean of at least 6 animals.

nificantly the effects of morphine (dose-ratio and 95% confidence limits 3.5; 2.6–6.3), but by this route was without effect on the antinociceptive actions of BW443C given subcutaneously (dose-ratio and 95% confidence limits 1.2; 0.9–1.8). N-methyl nalorphine (0.3 μg per mouse i.c.v.) also antagonized the effects of subcutaneous morphine in PBQ-induced writhing and slightly potentiated the effects of subcutaneous BW443C; the dose-ratio (and 95% confidence limits) for morphine was 3.7 (2.3–7.0) and for BW443C was 0.5 (0.4–0.7).

Effects on gastrointestinal propulsion

BW443C was a potent inhibitor of the propulsion of a charcoal meal in the mouse, the ED_{50} and 95% confidence limits being 3.2 (2.3–4.9) mg kg^{-1} s.c. For comparison, the potencies of morphine, pethidine and D-propoxyphene in this model were 1.1 (0.8–1.5), 19 (14–24) and 21 (17–26) mg kg^{-1}, respectively.

Discussion

In the present experiments, the novel opioid peptide H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2 (BW443C) displayed antinociceptive effects with a profile of activity different from that of the classical opiates morphine, pethidine and D-propoxyphene. Opioid μ-receptor agonists have been classified on the basis that their antinociceptive activity in a spectrum of animal models is independent of the type of nociceptor used e.g. chemical, heat or pressure stimuli (Martin *et al.*, 1976; Tyres, 1980). In the present study, such relative non-selectivity to stimuli was demonstrated in two chemically- (PBQ- and acetic acid-induced writhing) and two heat-induced (tailflick, hotplate) antinociceptive tests for morphine and, perhaps less convincingly for pethidine and D-propoxyphene. BW443C, however, demonstrated antinociceptive activity which was considerably greater in the chemical writhing assays compared with the heat-induced assays. In this

Table 2 Antagonist effects of naloxone and N-methyl nalorphine on inhibition of acetic acid-induced writhing by BW443C and morphine

	Dose-ratio				
	Naloxone (mg kg^{-1} i.p.)			*N-methyl nalorphine* (mg kg^{-1} i.p.)	
Agonist	3.75	7.5	7.5	11.5	15.0
BW443C	4.8* (1.3–21.4)	8.5** (2.1–33.1)	4.4** (2.1–10.0)		7.8** (3.5–16.8)
Morphine	2.9* (1.1– 8.7)	9.6** (3.6–29.3)		0.6 (0.3–1.0)	

Figures in parentheses are 95% confidence limits. *$P<0.05$, **$P<0.005$. At least 5 animals per dose group and a minimum of 4 doses were used for each ED_{50} determination.

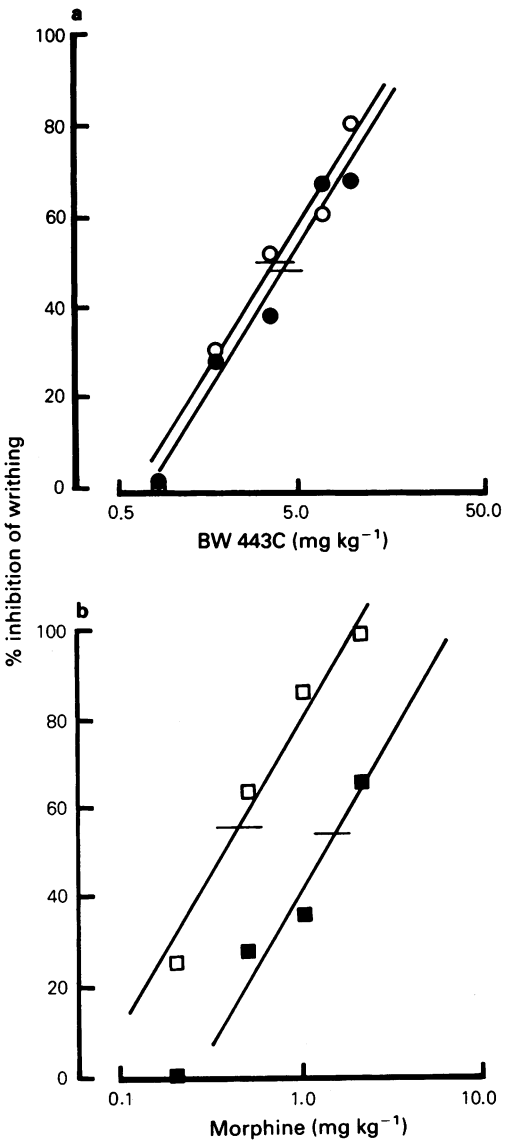

Figure 3 Inhibition of phenyl-*p*-benzoquinone-induced writhing responses in mice by (a) BW443C (s.c.) and (b) morphine (s.c.) in the absence (○, □) and presence (●, ■) of naloxone (i.c.v.) 0.3 μg per mouse. The horizontal bars indicate the 95% confidence limits.

respect, BW443C appears similar in antinociceptive profile to the opioid κ-receptor agonists such as buprenorphine and the benzomorphans (Tyers, 1980). That the activity of BW443C is κ-receptor mediated, however, appears to be unlikely. Firstly, the structurally closely related peptides H-Tyr-D-Met-Gly-Phe-Pro-NH_2 and its Phe^4 (4-NO_2) analogue have both been described as μ-agonists (Szekely *et al.*, 1977; Smith & Wilkinson, 1982) and secondly and more importantly, the opioid antagonist used in this study, N-methyl nalorphine acts preferentially at μ-receptor sites (Kobylecki *et al.*, 1982; Magnan *et al.*, 1982). Further, whereas κ-receptor agonists are only weak inhibitors of gastrointestinal propulsion compared with their antinociceptive activity (Hayes & Tyers, 1983; Ward & Takemori, 1983), BW443C and morphine were approximately equipotent inhibitors of gut propulsion and irritant-induced writhing, indicating predominant μ-receptor mediated effects for these two opioids. In contrast, pethidine and D-propoxyphene were markedly less potent in the gastrointestinal model than in antinociceptive writhing assays. The potency difference between the gastrointestinal and nociceptive models may reflect some κ-receptor mediated effects of these latter opioids, as described at least for D-propoxyphene by Neil & Terenius (1981) and suggested possibly by the relatively weak effects of pethidine in models of severe pain, such as the hotplate (Woolfe & Macdonald, 1944).

A different mode of action of BW443C from pethidine and D-propoxyphene was also indicated by their relative time courses in the noxious chemical- and heat-induced assays. Whereas for pethidine and D-propoxyphene a relatively simple dose-time relationship was observed in all four assays, an increased dose being required to produce equiactive effects with increased time after dosing, this relationship was observed for BW443C in the chemical writhing assays only. BW443C demonstrated a complex 'U'-shaped relationship in the hotplate and tailflick assays. Heat-induced nociception may be inhibited by opioids acting at supraspinal or spinal levels (Yaksh & Rudy, 1977), whereas chemical-induced writhing is less selective for opioid receptors (Tyers, 1980) and may detect antinociceptive effects of opioids mediated peripherally (Bentley *et al.*, 1981; Smith *et al.*, 1982; 1985). The high doses of BW443C required to produce antinociceptive effects in the hotplate and tailflick tests, therefore, may indicate that high circulating plasma levels are required in order to achieve low concentrations of BW443C in the CNS due to poor penetration of this polar material across the blood brain barrier (BBB). With time a slow accumulation of BW443C in the CNS may occur and be revealed as decreasing subcutaneous ED_{50} values. When the rate of elimination exceeds the rate of accumulation in the CNS, the ascending limb of the 'U'-shaped time course is observed. The absence of this complex 'U'-shaped function for BW443C in PBQ- and acetic acid-induced writhing may indicate, therefore, that there is no BBB situated between the subcutaneous site of administration and the site of action of BW443C in these models; that is, the antinociceptive effect of BW443C is

mediated peripherally. For lipophilic materials with an unrestricted CNS entry, a complex influence of the BBB on the dose-time course relationships may not be observed in any of the four antinociceptive models.

The antinociceptive activity of BW443C is qualitatively similar to that of the quaternary opioid agonist N-methyl morphine (Smith *et al.*, 1982). The similarity of the antinociceptive profiles of these opioids may result from the charged, lipophobic nature of these compounds which may provide for only very limited penetration of the BBB and thus promote opioid effects that are mediated peripherally.

The opioid nature of the antinociceptive effects of BW443C and morphine administered subcutaneously in acetic acid-induced writhing was confirmed by their antagonism by naloxone (3.75–7.5 mg kg^{-1} i.p). In this writhing model, however, BW443C-, but not morphine-induced antinociceptive effects were antagonized by the quaternary opioid antagonist N-methyl nalorphine (7.5–15 mg kg^{-1} i.p.) suggesting a peripheral mode of action for BW443C and a central one for morphine. Although quaternary narcotic antagonists have been used frequently in this way to localize the action of opioids, the important review by Brown & Goldberg (1985) highlights the dangers of interpreting experimental data with quaternary salts on the assumption of a selective peripheral action without the safeguard of a fuller pharmacological examination. Thus for example, although the absence of an effect of a quarternary antagonist after peripheral administration may be taken to imply a CNS effect of an agonist, on the assumption that no central penetration of the quaternary antagonist occurred, the lack of effect could be due to a low affinity of the quaternary antagonist at an obtained CNS site. Such alternative explanations, however, appear to be unlikely in the present experiments. Distribution studies of [^{14}C]-N-methyl nalorphine showed that after intravenous administration in mice minimal amounts of drug were observed in the brain at times of high plasma levels (Smith *et al.*, 1982; Parsons *et al.*, 1986). In addition, no demethylation to nalorphine was observed (unpublished observations).

In the present study, when access of N-methyl nalorphine to central receptors was enabled by intracerebroventricular injection, antagonism of morphine administered subcutaneously was observed. Other studies have also shown antagonism of morphine by N-methyl nalorphine when access to opioid receptors is available for the quaternary antagonist, for example *in vitro* in the electrically stimulated guinea-pig ileum (Kosterlitz & Waterfield, 1975; Smith *et al.*, 1982) and *in vivo* with local injections to the rat paw (Lorenzetti & Ferreira, 1982; Ferreira *et al.*, 1984). The inability of opioid antagonists administered intracerebroventicularly compared with intraperitoneally to antagonize the effects of subcutaneous BW443C in a writhing model, therefore, supports the hypothesis of a peripheral action of this peptide in its antinociceptive activity.

The demonstration using chemical- or pressure-induced nociceptive models of inhibitory effects on local administration of tertiary opioids and local or systemic administration of quaternary opioid agonists (Ferreira & Nakamura, 1979; Bentley *et al.*, 1981; Smith *et al.*, 1982) and antagonists (Lorezetti & Ferreira, 1982; Rios & Jacob, 1982; 1983) led to the concept of peripherally-mediated opioid antinociceptive mechanisms in models of acute inflammatory pain. The present results extend these observations to include the novel, polar opioid peptide, BW443C amongst the compounds exhibiting predominantly peripheral effects. Lorenzetti & Ferreira (1987) have also demonstrated antinociceptive effects of BW443C in a rat paw hyperalgesia model. Opioid effects in another peripheral model, neurogenic plasma extravasation, appear to be mediated by opioid receptors located on the peripheral terminals of small-diameter sensory neurones (Bartho & Szolcsanyi 1981; Smith & Buchan, 1984; Smith *et al.*, 1985). Such receptors could be involved in many peripherally-mediated opioid-induced effects by reducing sensory nerve activity. Thus, Russell *et al.* (1987) have demonstrated recently that opioids such as morphine and ethylketocyclazocine significantly inhibited spontaneous discharges from small-diameter afferent fibres from inflamed knee joints of the cat. Likewise, in the vagus, BW443C has been demonstrated to reduce spontaneous impulse activity in Aδ-fibres, an effect possibly correlated with the antitussive activity of the compound (Adcock *et al.*, 1987). It is hypothesised, therefore, that peripherally-mediated, opioid-induced antinociceptive effects may be mediated by inhibition of sensory neurones thus preventing information about the noxious events in the peritoneum for example from reaching the spinal cord.

References

ADCOCK, J.J., ALLAN, G., RICHARDSON, P.J., SCHNEIDER, C. & SMITH T.W. (1987). Antitussive effects and inhibition of vagal sensory nerve activity by Tyr-D-Arg-Gly-Phe$_4$ (4-NO_2)-Pro-NH_2) (443C). *Br. J. Pharmacol. Proc. Suppl.*, **90**, 143P.

BAJUSZ, S., RONAI, A.Z., SZEKELY, J.I., GRAF, L., DUNAI-KOVACS, Z. & BERZETEI, I. (1977). A superactive antinociceptive pentapeptide, (D-Met^2, Pro^5)-enkephalinamide. *Febs Letts.*, **76**, 91–92.

BARTHO, L. & SZOLCSANYI, J. (1981). Opiate agonists inhibit neurogenic plasma extravasation in the rat. *Eur. J. Pharmacol.*, **73**, 101–104.

BENTLEY, G.A., NEWTON, S.H. & STARR, J. (1981). Evidence for an action of morphine and the enkephalins on sensory nerve endings in the mouse peritoneum. *Br. J. Pharmacol.*, **73**, 325–332.

BIANCHI, G., FIOCCHI, R., TAVANI, A. & MANARA, L. (1982). Quaternary narcotic antagonists relative ability to prevent antinociception and gastrointestinal transit inhibition in morphine-treated rats as an index of peripheral selectivity. *Life Sci.*, **30**, 1875–1882.

BROWN, D.R. & GOLDBERG, L.I. (1985). The use of quaternary narcotic antagonists in opiate research. *Neuropharmacol.*, **24**, 181–191.

FERREIRA, S.J., LORENZETTI, B.B. & RAE, G.A. (1984). Is methyl nalorphinium the prototype of an ideal peripheral analgesic? *Eur. J. Pharmacol.*, **99**, 23–29.

FERREIRA, S.J., MOLINA, N. & VETTORE, O. (1981). Prostaglandin hyperalgersia V: a peripheral analgesic receptors for opiates. *Prostaglandins*, **23**, 53–58.

FERREIRA, S.H. & NAKAMURA, M. (1979). II-Prostaglandin hyperalgesia: the peripheral analgesic activity of morphine, enkephalins and opioid antagonists. *Prostaglandins*, **18**, 191–200.

FOLLENFANT, R.L., HARDY, G.W., LOWE, L.A. & SMITH, T.W. (1987). Antinociceptive effects of a novel peripherally acting opioid peptide Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2 (443C). *Br. J. Pharmacol Proc. Suppl.*, **90**, 68P.

GREEN, A.F. (1959). Comparative effects of analgesics on pain threshold, respiratory frequency and gastrointestinal propulsion. *Br. J. Pharmacol Chemother.*, **14**, 26–34.

GREEN. A.F., YOUNG, P.A. & GODFREY, E.I. (1951). A comparison of heat and pressure analgesiometric methods in the rat. *Br. J. Pharmacol Chemother.*, **6**, 572–585.

HARDY, G.W., LOWE, L.A. & SMITH, T.W. (1987). Enkephalin analogues containing polar amino-acid residues. In *Peptides 1986, Proc. 19th Eur. Pep. Symp.*, ed. Theodoropoulos, D. pp. 435–438 Berlin: Walter de Gryter.

HAYES, A.G. & TYERS, M.B. (1983). Determination of receptors that mediated opiate side effects in the mouse. *Br. J. Pharmacol..*, **79**, 731–736.

KOBYLECKI, R.J., LANE, A.C., SMITH, C.F.C., WAKELIN, P.L.G., CRUSE, W.B.T., EGERT, E. & KENNARD, O. (1982). N-methylnalorphine: definition of N-allyl conformation for antagonism at the opiate receptor. *J. Med. Chem.*, **25**, 1278–1280.

KOSTERLITZ, H.W. & WATERFIELD, A.A. (1975). *In vitro* models in the study of structure-activity relationships of narcotic analgesics. *Ann. Rev. Pharmacol., Toxicol.*, **15**, 29–47.

LORENZETTI, B.B. & FERREIRA, S.H. (1982). The analgesic effect of quaternary analogues of morphine and nalorphine. *Braz. J. Med. Biol. Res.*, **15**, 285–290.

LORENZETTI, B.B. & FERREIRA, S.H. (1987). On the mode of analgesic action of Tyr.D.Arg.Gly.Phe(4-NO_2). Pro.NH_2 (443C). *Br. J. Pharmacol. Proc. Suppl.*, **90**, 69P.

MAGNAN, J., PATERSON, S.J., TAVANI, A. & KOSTERLITZ, H.W. (1982). The binding spectrum of narcotic analgesic drugs with different agonist and antagonists properties. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **319**, 197–205.

MARTIN, W.R., EADES, C.G., THOMPSON, J.A., HUPPLER, R.E. & GILBERT, P.E. (1976). The effect of morphine- and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.*, **154**, 497–505.

NEIL. A. & TERENIUS, L. (1981). D-propoxyphene acts differently from morphine on opioid receptor-effector mechanisms. *Eur. J. Pharmacol.*, **69**, 33–39.

PARSONS, D.N., SCHNEIDER, C. & SMITH, T.W. (1986). Peripheral antitussive action of codeine and morphine in the guinea-pig. *Br. J. Pharmacol. Proc. Suppl.*, **99**, 818P.

RIOS, L. & JACOB, J.J.C. (1982). Inhibition of inflammatory pain by naloxone and its N-methyl quaternary analogue. *Life Sci.*, **31**, 1209–1212.

RIOS, L. & JACOB, J.J.C. (1983). Local inhibition of inflammatory pain by naloxone and its N-methyl quaternary analogue. *Eur. J. Pharmacol.*, **96**, 277–283.

RUSSELL, J., BASS, P., GOLDBERG, L.I., SCHUSTER, C.R. & MERZ, H. (1982). Antagonism of gut, but not central effects of morphine with quaternary narcotic antagonists. *Eur. J. Pharmacol.*, **78**, 255–261.

RUSSELL, N.J.W., SCHAIBLE, H.-G & SCHMIDT, R.F. (1987). Opiates inhibit the discharges of fine afferent units from inflamed knee joint of the cat. *Neurosci. Lett.*, **76**, 107–112.

SMITH, T.W. & BUCHAN, P. (1984). Peripheral opioid receptors located on the rat saphenous nerve. *Neuropeptides*, **5**, 217–220.

SMITH, T.W., BUCHAN, P., PARSONS, D.N. & WILKINSON, S. (1982). Peripheral antinociceptive effects of N-methyl morphine. *Life Sci.*, **31**, 1205–1208.

SMITH, T.W., FOLLENFANT, R.L. & FERREIRA, S.H. (1985). Antinociceptive models displaying peripheral opioid activity. *Int. J. Tiss. Reac.*, **7**, 61–67.

SMITH, T.W. & WILKINSON, S. (1982). The chemistry and pharmacology of opioid peptides. In *The Chemical Regulation of Biological Mechanisms*. ed. Creighton, A.M. & Turner, S. pp. 231–254. London; Royal Society of Chemistry.

SZEKELY, J.I., RONAI, A.Z., DUNAI-KOVACS, Z., MIGLECZ, E., BERZETEI, I., BAJUSZ, S. & GRAF, L. (1977). (D.Met^2, Pro^5)-enkephalinamide: a potent morphine-like analgesic. *Eur. J. Pharmacol.*, **43**, 293–294.

TYERS, M. B. (1980). A classification of opiate receptors that mediate antinociception in animals. *Br. J. Pharmacol.*, **69**, 503–512.

WARD, S.J. & TAKEMORI, A.E. (1983). Relative involvement of receptor subtypes in opioid-induced inhibition of gastrointestinal transmit in mice. *J. Pharmacol. Exp. Ther.*, **224**, 359–363.

WOOLFE, G. & MACDONALD, A. (1944). The evaluation of the analgesic action of pethidine hydrochloride (demoral). *J. Pharmacol. Exp. Ther.*, **80**, 300–307.

YAKSH, T.L. & RUDY, T.A. (1977). Studies on the direct spinal action of narcotics on the production of analgesia in the rat. *J. Pharmacol. Exp. Ther.*, **202**, 411–428.

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