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Effects of codeine, morphine and a novel opioid pentapeptide BW443C, on cough, nociception and ventilation in the unanaesthetized guinea-pig

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1 Antitussive, antinociceptive and respiratory depressant effects of codeine, morphine and H.Tyr.D-Arg.Gly.Phe(4-NO_2) Pro.NH_2 (compound BW443C) were investigated in unanaesthetized guinea-pigs. Antagonism of the antitussive and antinociceptive effects was investigated by the use of nalorphine and N-methylnalorphine. Naloxone was used to antagonize respiratory depression.

2 Antitussive ED_{50}s (with 95% confidence limits) for inhibition of cough induced by citric acid vapour were for codeine, morphine and BW443C respectively, 9.1(5.8–15), 1.3(0.7–2.4) and 1.2(0.6–2.6) mg kg^{-1} s.c. and 8.7(4.2–12), 1.6(1.2–1.9) and 0.67(0.002–3.3) mg kg^{-1}, i.v. The antitussive effects of subcutaneous codeine (25 mg kg^{-1}) morphine (8.1 mg kg^{-1}) and BW443C (2.5 mg kg^{-1}) were significantly antagonized by subcutaneous nalorphine (3.0 mg kg^{-1}) and N-methylnalorphine (3.0 mg kg^{-1}).

3 In the multiple toe-pinch test, the antinociceptive ED_{50}s (with 95% confidence limits) of codeine and morphine were 18(16–22) and 2.3(0.4–4.3) mg kg^{-1}, s.c., respectively. Compound BW443C was ineffective in doses of 2.5 and 10 mg kg^{-1} s.c., a result consistent with its lacking penetration into the CNS. Subcutaneous nalorphine (3.0 mg kg^{-1}) antagonized the antinociceptive action of codeine (25 mg kg^{-1}) and morphine (8.1 mg kg^{-1}). In contrast, N-methylnalorphine (3.0 mg kg^{-1}) had no significant effect on the antinociceptive action of codeine and morphine, suggesting lack of penetration of the CNS by N-methylnalorphine.

4 At doses near to the i.v. ED_{50} values for the antitussive activity, morphine (1.5 mg kg^{-1}, i.v.) and codeine (10 mg kg^{-1}, i.v.) caused small but significant depressions of ventilation (7.0 ± 2.3% and 16.5 ± 8.4% respectively). Higher doses of morphine (10, 30 and 60 mg kg^{-1}, i.v.) caused further dose-related depression of ventilation (9.6 ± 5.3%, 22.4 ± 6.2% and 36.2 ± 9.6% respectively) whereas codeine (30 and 60 mg kg^{-1} i.v.) caused stimulation of ventilation which was marked (191.3 ± 43.9%) at 60 mg kg^{-1}.

5 Compound BW443C in doses of 1 or 10 mg kg^{-1}, i.v. (approximately equal to, and 10 times the ED_{50} for antitussive activity) did not cause significant depression of ventilation. Only at higher doses of 30 and 60 mg kg^{-1}, i.v. was there a significant decrease in minute volume (13.1 ± 6.8% and 15.9 ± 1.89% respectively). The depression of ventilation caused by either BW443C (60 mg kg^{-1}, i.v.) or morphine (60 mg kg^{-1}, i.v.) was prevented by pretreatment with naloxone (3 mg kg^{-1}, i.v.) administered 15 min before morphine or BW443C.

6 These results in the guinea-pig support the hypothesis that the antitussive action of the opiates codeine and morphine and the opioid pentapeptide BW443C do not require penetration of these drugs into the CNS.

Introduction

In addition to analgesic actions, opiates such as morphine and codeine are well known antitussive agents. Like opioid-induced analgesia, opioid-induced antitussive effects are assumed generally to be mediated centrally (Eddy *et al.*, 1969; Salem & Aviado, 1970), although some peripheral components of the antitussive effects have been postulated (Yanaura *et al.*, 1981). In a recent study (Follenfant *et al.*, 1987) in this laboratory, antinociceptive effects of a novel, polar enkephalin analogue H.Tyr.D-Arg.Gly.Phe(4-NO_2).Pro.NH_2 (BW443C) have been demonstrated in support of the hypothesis that opioids with restricted penetration of the blood brain barrier may induce peripherally-mediated antinociceptive effects (Smith

& Clark, 1982; 1985; Lorenzetti & Ferreira, 1982). In the present experiments we have extended the study with BW443C to explore whether peripherally-mediated opioid antitussive effects also may be observed. Antitussive actions of BW443C, morphine and codeine and their inhibition by the quarternary opioid antagonist N-methylnalorphine have been investigated in the unanaesthetized guinea-pig. In comparison, the depressant effects of BW443C, morphine and codeine on respiratory ventilation in the unanaesthetized guinea-pig have also been investigated.

Methods

Antitussive tests

The method used was based on that described by Boura *et al.* (1970). Male albino Dunkin-Hartley strain guinea-pigs (377–557 g) were used in groups of four. The animals were deprived of access to food and water for 30 min from before intravenous drug treatment until after aerosol challenge. Graded doses of BW443C, morphine, codeine or saline were injected in a dose volume of 0.25 ml kg^{-1} into an ear vein; 5–15 min later, each of the four guinea-pigs was placed in a 20 litre glass container (in a fume cupboard) to acclimatize for 15 min. Citric acid vapour from a Collison inhaler was than introduced for 5 min into the vessel followed 2.5 min later by compressed air (7 litre min^{-1}) to dilute steadily the citric acid vapour. Coughs were recorded by a microphone placed in each chamber and monitored via a pre-amplifier to a loudspeaker and cassette deck. The number of coughs occurring within a 12.5 min period, beginning 20–30 min after intravenous injection at each dose was used to calculate ED_{50} s; the ED_{50} being defined as that dose of drug which reduced by 50% the number of coughs in the drug-treated group compared with the number of coughs in saline-treated controls on exposure to the citric acid vapour. When drugs were given subcutaneously, injections were made 30 min before citric acid challenge. In antagonist experiments, nalorphine or N-methyl nalorphine was given 15 min before challenge. In all experiments, doses were coded to eliminate observer bias and all animals were used once only.

Antinociceptive tests

The method used was based on that described by Collier *et al.* (1961). Animals were similar to those described above. Opioids were given subcutaneously in dose volumes of 5 ml kg^{-1}, 30 or 60 min before application of the noxious stimulus which consisted of the application of an artery clip for approximately one second to all 14 or 8 fore toes as indicated in Table 2. A squeak on application of the clip to a toe was recorded as a positive nociceptive response. Nalorphine or N-methylnalorphine was given subcutaneously 15 or 30 min before the nociceptive challenge. Animals were used once only.

Measurement of ventilation in conscious guinea-pigs

Male Dunkin-Hartley guinea-pigs (250–350 g) were anaesthetized with 2.5% halothane (carrier gases: O_2 1 litre min^{-1}, N_2O 1 litre min^{-1}). A small incision was made in the right upper forelimb and the brachial vein was cannulated with polyethylene tubing (pp10) to a length of approximately 2 cm. At least 20 cm of tubing, containing heparinised saline (125 u ml^{-1}), was left outside the incision. The wound was then swabbed with ethanol and closed with three sutures. Following application of Nobecutane to the sutured area, the remaining 20 cm of tubing was wound in a coil and taped to the front limb. Animals were allowed to recover for 24 h.

The plethysmograph was modified from the method of Amdur & Mead (1958) and consisted of three pieces; a reservoir bottle, a connector piece and an animal chamber. Guinea-pigs were placed in the airtight plethysmograph chamber and held in position by a secure plastic neck piece. The base of the chamber was lined with foam rubber to prevent the animals from sliding on the perspex during an experiment. A small latex collar was introduced over the animal's head and smoothed against the plethysmograph surface. Honey was used as a sealant throughout. An outer latex collar was placed over the inner collar and around the plethysmograph to obtain an airtight seal; a small hole being cut in the outer collar for exteriorising the implanted cannula tubing.

The plethysmograph chamber was attached to a 5 litre reservoir bottle (filled with copper gauze to provide a heat sink) via the connecting piece. A Validyne differential pressure transducer (MP45) was connected to the plethysmograph. The complete unit was positioned in a polystyrene-lined cabinet. Breath-by-breath recording via a Buxco (model 6) pulmonary mechanics analyser of the ventilatory parameters, flow, tidal volume, respiratory rate and minute volume were displayed in analogue form on a Beckman R611 dynograph recorder and also in digital form on a Texas Instruments data terminal (silent 700) via a Buxco data logger (model DC-12).

In preliminary control experiments it was found that during the first hour of recording, animals were restless and ventilation irregular. However, during the second, third and fourth hours animals settled down and ventilation became regular throughout this period. Thus a rigid protocol was adopted for each animal; ventilatory parameters were recorded for a

period of 2 h before and 1.5 h after administration of vehicle or test drug. All drugs were administered intravenously as single bolus injection via the implanted brachial cannula in a dose volume of 1 ml kg^{-1}.

Statistical analysis

In antitussive and antinociceptive tests ED_{50} values with 95% confidence limits were calculated by linear regression analysis. *P* values, based on analysis of variance, were calculated by the Mann-Whitney U test. Thus median doses in addition to means ± s.e.mean were calculated.

In tests on ventilation the mean values of 30 min breath-by-breath analysis (averaged every minute by the data logger) before vehicle or drug treatment were compared to the mean values of 30 min analysis in the presence of vehicle or drug by Student's *t* test. In addition, mean % changes in minute volume in groups of animals in the presence of drug were compared to the mean % changes in minute volume in groups of animals that had received saline. Statistical significance was assessed by Student's *t* test.

Materials

Codeine phosphate, morphine hydrochloride and nalorphine hydrobromide were supplied by The Wellcome Foundation. N-methylnalorphine hydrochloride and H.Tyr.D-Arg.Gly.Phe(4-NO_2).Pro.NH_2 diacetate (compound BW443C) were supplied by Dr S. Wilkinson and Mr L.A. Lowe, respectively, of the Medicinal Chemistry Department, Wellcome Research Laboratories, Beckenham, Kent. Citric acid (Analar) was purchased from BDH Chemicals Ltd., Poole, Dorset; Halothane, May and Baker; heparin sodium injection, Duncan Flockhart and Co. Ltd., honey, commercial brand; liquid latex, General Latex, Cambridge MA (formulation 1-N-62); Nobecutane, Astra Chemicals Ltd; naloxone hydrochloride, Endo Laboratories; pentobarbitone forte, Veterinary Drug Co., PLC; and sodium chloride 0.9% w/v (steriflex), from Boots Company Ltd. The anticoagulant consisted of the following chemicals: absolute ethanol (58.5 g), BDH; calcium nitrate.4H_2O (40.75 g) BDH; Igepal CA-630 (0.75 g), Gaf Chemicals; made up to 200 ml with distilled water. All drugs were dissolved in 0.9% w/v solution of sodium chloride (saline). Doses are expressed as the free base.

Results

Antitussive tests

The antitussive ED_{50}s (with 95% confidence limits) for inhibition of cough induced by citric acid vapour for codeine, morphine and BW443C were 9.1(5.8–15), 1.3(0.7–2.4) and 1.2(0.6–2.6) mg kg^{-1} s.c. respectively and 8.7(4.2–12), 1.6(1.2–1.9) and 0.67(0.002–3.3) mg kg^{-1} i.v. respectively. Thus codeine and morphine were as potent s.c. as they were i.v., whereas BW443C was about twice as potent i.v. as it was s.c. Compound BW443C was at least as potent as morphine by either route.

The effects of given combinations of antitussive drugs and opioid antagonists in four separate experiments are summarised in Table 1. Although codeine (25 mg kg^{-1}, s.c.) had a significant antitussive effect ($P < 0.02$) at 30 min, this did not occur in guinea-pigs also given N-methynalorphine (3.0 mg kg^{-1}) 15 min after codeine. In another experiment there were significantly more coughs in animals given morphine (8.1 mg kg^{-1}) plus nalorphine (3.0 mg kg^{-1}) or N-methylnalorphine (3.0 mg kg^{-1}) than in animals given morphine plus saline before citric acid challenge. The antitussive action of morphine appeared to be antagonized by nalorphine or N-methylnalorphine to about the same extent. In a third experiment, animals given codeine plus nalorphine or codeine plus N-methylnalorphine coughed significantly more times than their corresponding controls given codeine plus saline ($P = 0.015$ and 0.005 respectively). The last experiment summarized in Table 1 shows that animals given BW443C (2.5 mg kg^{-1}) coughed significantly ($P = 0.002$) less than saline-treated controls when challenged by citric acid, but this antitussive action of BW443C was antagonized by N-methylnalorphine.

Antinociceptive tests

In the multiple toe-pinch test, codeine given 30 min before challenge had an antinociceptive ED_{50} of 18(16–22) mg kg^{-1}, s.c. In a similar test, morphine at 60 min had an ED_{50} of 2.3(0.4–4.3) mg kg^{-1}, s.c. In another experiment, animals given codeine, 25 mg kg^{-1}, s.c. at 15 or 30 min before nociceptive challenge, responded 47% ($P = 0.023$) and 70% ($P = 0.008$) respectively less than saline-treated animals. However, BW443C in doses of 2.5 or 10 mg kg^{-1}, s.c. was ineffective at either 15 or 30 min.

Table 2 summarizes three experiments in which antagonism of the opiates by N-methylnalorphine and nalorphine was investigated. In the first experiment, the antinociceptive action of codeine was not antagonized by N-methylnalorphine, as shown by the fact that animals given codeine plus saline or codeine plus N-methylnalorphine, showed significantly fewer nociceptive responses than animals given saline alone.

In another experiment, guinea-pigs given morphine 8.1 mg kg^{-1}, s.c., 60 min before challenge, plus nalorphine, 3.0 mg kg^{-1}, s.c. 30 min later, showed significantly ($P = 0.003$) more nociceptive responses

Table 1 Effects of drug combinations on cough induced by citric acid vapour

Treatment s.c. *First*	*Dose* ($mg\,kg^{-1}$)	*Second*	*Dose* ($mg\,kg^{-1}$)	n	*Mean no. of coughs*	*Median no. of coughs*	P *value**
Saline	—	Saline	—	6	14 ± 3.5	10.5	—
Codeine	25	Saline	—	6	1.8 ± 1.4	0.5	0.0152
Saline	—	N-methylnalorphine	3.0	6	12 ± 2.5	13.0	NS
Codeine	25	N-methylnalorphine	3.0	6	14 ± 3.8	16.5	NS
Morphine	8.1	Saline	—	8	0.4 ± 0.4	0	—
Morphine	8.1	Nalorphine	3.0	8	6.8 ± 2.7	3.0	0.0281
Morphine	8.1	N-methylnalorphine	3.0	8	5.5 ± 2.4	2.0	0.0379
Codeine	25	Saline	—	8	4.5 ± 3.6	0	—
Codeine	25	Nalorphine	3.0	8	14 ± 4.8	12.5	0.0148
Codeine	25	Saline	—	8	0.5 ± 0.3	0	—
Codeine	25	N-methylnalorphine	3.0	8	9.8 ± 3.0	7.0	0.0047
Saline	—	Saline	—	6	19 ± 2.6	19.5	—
BW443C	3.0	Saline	—	6	5.7 ± 1.0	6.5	0.0022
Saline	—	N-methylnalorphine	3.0	6	15 ± 1.9	14.0	NS
BW443C	3.0	N-methylnalorphine	3.0	6	14 ± 1.4	13.0	NS

The first treatment saline, codeine 25 $mg\,kg^{-1}$, morphine 8.1 $mg\,kg^{-1}$ or BW443C 3 $mg\,kg^{-1}$ was given 30 min before challenge with citric acid vapour generated from a 30% w/v solution. The second treatment, saline, nalorphine 3.0 $mg\,kg^{-1}$, or N-methylnalorphine 3.0 $mg\,kg^{-1}$ was given 15 min before challenge. *n*, no. of guinea-pigs. NS, not statistically significant ($P > 0.05$). Coughs were counted for 12.5 min after start of challenge: mean no. of coughs ± s.e.mean are shown. All drugs were given subcutaneously, as soluble salts in saline. Doses refer to active base.
*Mann-Whitney U test.

than guinea-pigs given morphine plus saline, indicating that nalorphine had antagonized the antinociceptive action of morphine. As animals given morphine plus N-methylnalorphine, did not respond significantly more than those given morphine plus saline, N-methylnalorphine did not appear to antagonize morphine in this test.

In a similar experiment, codeine 25 $mg\,kg^{-1}$, s.c.,

Table 2 Effects of drug combinations on vocalization to nociceptive challenge in the guinea-pig multiple toe-pinch test

Treatment s.c. *First*	*Dose* ($mg\,kg^{-1}$)	*Second*	*Dose* $mg\,kg^{-1}$	n	*No. of toes challenged per animal*	*Mean no. of squeaks per animal*	*Median no. of squeaks per animal*	*value**
Saline	—	Saline	—	6	14	13 ± 0.4	14.0	—
Codeine	25	Saline	—	7	14	6.1 ± 0.1	3.0	0.0006
Saline	—	N-methylnalorphine	3.0	6	14	14 ± 1.5	14.0	NS
Codeine	25	N-methylnalorphine	3.0	7	14	4.1 ± 1.4	3.0	0.0003
Morphine	8.1	Saline		8	8	1.1 ± 0.7	0	—
Morphine	8.1	Nalorphine		8	8	5.6 ± 0.8	6.0	0.003
Morphine	8.1	N-methylnalorphine		8	8	2.6 ± 1.1	1.5	NS
Codeine	25	Saline		10	8	1.9 ± 0.7	1.0	—
Codeine	25	Nalorphine		9	8	5.1 ± 1.0	6.0	0.0279
Codeine	25	N-methylnalorphine		8	8	1.8 ± 1.0	0	NS

Drug treatments were as described in Table 1 except that morphine was given 60 min before nociceptive challenge and nalorphine or N-methylnalorphine given 30 min after morphine. Challenge was the application to each toe for approximately 1 s, of an artery clip. One squeak was counted as a positive response. Mean no. of squeaks ± s.e.mean are shown. *n*, no. of animals.
*Mann-Whitney U test. Other details as in Table 1.

given 30 min before challenge appeared to be significantly antagonized ($P = 0.028$) by nalorphine, 3.0 mg kg^{-1}, s.c., given 15 min later but not by N-methylnalorphine, given at the same dose at a comparable time.

Effects of morphine, codeine and BW443C on ventilation

Intravenous administration of morphine to conscious guinea-pigs, in the head out body plethysmograph box, at 1.5 mg kg^{-1} (approximating the i.v. ED_{50} dose for antitussive activity in the same species), 10, 30 and 60 mg kg^{-1}, i.v., resulted in statistically significant depression of ventilation, as measured by % decrease in minute volume which was dose-related (Figure 1). On examination of the absolute values of the individual parameters measured (peak expiratory flow, tidal volume, respiratory rate, minute volume) it was observed that the ventilatory depression induced by intravenous administration of morphine was due predominantly to a decrease in tidal volume. There was little or no effect on the respiratory rate except after the highest dose (60 mg kg^{-1}, i.v.).

When administered intravenously to conscious guinea-pigs, in the head out body plethysmograph box, codeine at 10 mg kg^{-1} (approximately equal to i.v. ED_{50} dose for antitussive activity in the same species) also resulted in a statistically significant (16.5 ± 8.4%) depression of ventilation as measured by a % decrease in minute volume (Figure 2). In

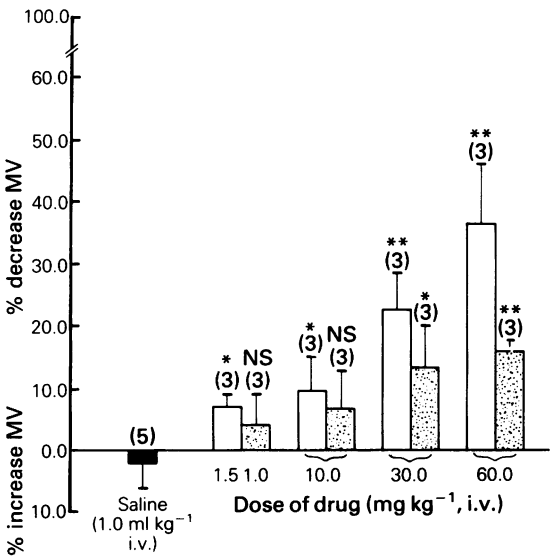

Figure 1 The effect of intravenous administration of saline (solid column), morphine (open columns) and BW443C (stippled columns) on ventilation (expressed as % change in minute volume (MV) of 30 min analysis in the presence of treatment from 30 min analysis before treatment) in conscious guinea-pigs in the head out body plethysmograph box. Drugs were administered as single bolus injections in saline (dose volume 1 ml kg^{-1}) and expressed as mg kg^{-1}. Numbers in parentheses indicate numbers of animals per treatment and vertical bars show s.e.mean. Values in the presence of drug treatment were compared with those in the presence of saline by Student's t test. Values which are significantly different are shown by $*P < 0.05$; $**P < 0.005$, NS = not significant. Other details in Table 1.

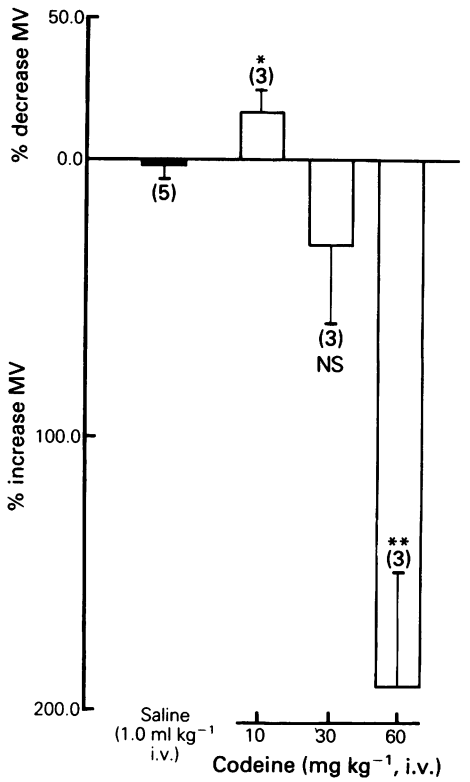

Figure 2 The effect of intravenous administration of saline (solid column) or codeine (open columns) on ventilation (expressed as % change in minute volume (MV) of 30 min analysis in the presence of treatment from 30 min analysis before treatment) in conscious guinea-pigs in the head out body plethysmograph box. Codeine was administered as single bolus injection in saline (dose volume 1 ml kg^{-1}) and expressed as mg kg^{-1}. Figures in parentheses indicate numbers of animals per treatment and vertical bars show s.e.mean. Values in the presence of codeine were compared with those in the presence of saline by Student's t test. Values which are significantly different are shown by $*P < 0.05$; $**P < 0.005$, NS = not significant. Other details as in Table 1.

contrast to morphine, this effect of codeine was due predominantly to a reduction in respiratory rate with little or no effect on the tidal volume. At higher doses 30 and 60 mg kg^{-1}, i.v., codeine produced stimulation of ventilation which was marked (191.3 ± 43.9%) after 60 mg kg^{-1}. The absolute values for peak expiratory flow, tidal volume, respiratory rate and minute volume were all substantially increased following this dose.

In contrast to morphine and codeine, BW443C at 1.0 mg kg^{-1} (approximately equal to the i.v. ED_{50} dose of antitussive activity in the same species) did not produce significant depression of ventilation (Figure 1). In addition, although BW443C at 10 mg kg^{-1}, i.v., induced a significant fall in respiratory rate there was also a significant increase in tidal volume resulting in a net effect of a small (6.7 ± 5.8%) but not significant depression of ventilation (as measured by % decrease in minute volume). Only at the higher doses of BW443C (30 and 60 mg kg^{-1}, i.v.) was there a significant % decrease in minute volume (13.1 ± 6.8 and 15.9 ± 1.89 respectively). Depression of ventilation at these doses was due to a significant decrease in respiratory rate and although compensation in the form of an increase in tidal volume occurred it was not sufficient to offset the effect on rate.

Saline 1 ml kg^{-1}, i.v., or naloxone 3 mg kg^{-1}, i.v., was administered as a single bolus injection 15 min before morphine (60 mg kg^{-1}, i.v.) or BW443C (60 mg kg^{-1}, i.v.). Whereas naloxone had virtually no effect by itself, the decrease in minute volume caused by morphine was reduced from 31.6 ± 8.6% (guinea-pigs predosed with saline) to 4.0 ± 8.4% in the presence of naloxone. Similar results were obtained with BW443C (60 mg kg^{-1}, i.v.) in which the decrease in minute volume caused by BW443C was reduced from 13.5 ± 2% (guinea-pigs predosed with saline) to 1.03 ± 4.9% in the presence of naloxone.

Discussion

The argument that the enkephalin analogue, H.Tyr.D-Arg.Gly.Phe(4-NO_2)Pro.NH_2 (compound BW443C) has a peripheral rather than a central antitussive action in the guinea-pig, is based on the following evidence. First, its antitussive action (at 2.5 mg kg^{-1}) was reduced significantly by N-methylnalorphine, which being a quaternary salt of nalorphine, was designed not to penetrate the blood-brain barrier, and which has been shown in mice and guinea-pigs to be excluded from the CNS (Parsons *et al.*, 1986; see also Brown & Goldberg, 1985). Second, whole body radiographic studies in the rat, have shown (Parsons, personal communication) that there was little BW443C in the brain compared to plasma levels within 45 min of an intravenous injection of 7.8 mg kg^{-1}. Third, BW443C unlike codeine or morphine, was ineffective in the guinea-pig multiple toe-pinch test, which was reported by Collier *et al.* (1961) to be specifically sensitive to the antinociceptive action of opiates such as codeine and morphine. This lack of antinociceptive activity in models of acute moderate pain is consistent with results in mice reported by Follenfant *et al.* (1987). Fourth, as shown by experiments described in this paper, BW443C in i.v. doses near to or approximately 10 times its antitussive ED_{50} did not cause significant depression of ventilation, in contrast to the depressant effects caused by codeine and morphine.

The fact that N-methylnalorphine (3 mg kg^{-1}, s.c.) antagonized the antitussive action of codeine (25 mg kg^{-1}) and morphine (8.1 mg kg^{-1}), but had no effect on the antinociceptive action of either codeine or morphine in the guinea-pig, leads us to believe that the antitussive action of these opiates is not centrally mediated, in this model. Although earlier reviews (Eddy *et al.*, 1969; Salem & Aviado, 1970) refer to the central antitussive action of codeine and other opiates, Jaffe & Martin (1985) concluded that the exact mechanism is still not entirely clear. Antitussive activity of quaternary salts of codeine and morphine has not been reported. Chakravarty *et al.* (1956) showed that in decerebrate cats, in which coughs were elicited by electrical stimulation of the dorsolateral region of the medulla, codeine in doses of 4 to 10 mg kg^{-1} i.v. in 7 experiments depressed the frequency and intensity of cough.

One alternative explanation to our results could be that the guinea-pig models used, codeine, morphine and BW443C mediate (in very low concentrations) central antitussive actions, at opioid receptors which differ from those mediating antinociceptive effects; and that N-methylnalorphine (also in very low concentrations) antagonizes these opioids only at the 'antitussive' receptors. It is well known that dextromethorphan (which was also reported by Chakravarty *et al.* (1956), to have, like codeine, a central mode of action) reduces cough but has no analgesic action. To date, however there is no direct experimental evidence to support the belief that there are specific 'antitussive' opioid receptors in the 'cough centre' of the medulla. The evidence that electrical stimulation of the medulla induces cough, that can be suppressed by opiates, does not prove that pathological cough is inhibited exclusively by central opioid action. There is no evidence suggesting that the antitussive action of codeine or morphine is due to a local anaesthetic effect; experiments in our laboratory have shown that BW443C does not have local anaesthetic activity. Pickering & James (1979a,b) reported on the antitussive activity and pharmacology of a novel compound RU 20201, which appeared to have a peripheral mode of action in comparative tests using codeine as

reference. However, unlike BW443C, there was no evidence that RU20201 was an opioid-like drug.

Yanaura *et al.* (1981) have claimed from their experiments in dogs that codeine and morphine have a peripheral mode of action, in addition to a central antitussive action. Recent experiments in our laboratory (Adcock *et al.*, 1987) have indicated that in anaesthetized cats, at least part of the antitussive activity of BW443C may be due to inhibition of afferent sensory nerve endings in the respiratory tract.

Both codeine ($10\,mg\,kg^{-1}$, i.v.) and morphine ($1.5\,mg\,kg^{-1}$, i.v.) at doses near to their i.v. ED_{50} doses for antitussive activity caused statistically significant depression of ventilation as measured by a % decrease in minute volume. However, the mechanism by which this depression occurred at these doses differed. Morphine appeared to affect tidal volume predominantly without affecting respiratory rate; suggesting that it was influencing primarily the sensitivity of the tidal volume-CO_2 controller in the medulla thus making it less sensitive to changes in PCO_2 levels. On the other hand, administration of codeine reduced the respiratory rate with little or no effect on tidal volume. The fact that there was no compensation for the decrease in respiratory rate suggests that codeine also affected the sensitivity of the tidal volume-CO_2 controller to PCO_2 levels. After doses higher than the antitussive ED_{50} (i.v.) doses the two drugs differed again. Morphine caused a further dose-related depression of ventilation with a $36.2 \pm 9.6\%$ decrease in minute volume occurring at $60\,mg\,kg^{-1}$, i.v. In contrast, codeine produced stimulation of ventilation with a $191.3 \pm 43.9\%$ increase in minute volume occurring at $60\,mg\,kg^{-1}$, i.v. The reason for this stimulation by codeine remains unknown but it does not apparently occur when administered subcutaneously. It has, however, been observed in other species when administered intravenously e.g. the rabbit (Mayor & Wiki, 1911, Furukawa & Okabe, 1958). Whether or not it is due to an opiate receptor mechanism remains to be elucidated.

In contrast to morphine and codeine, BW443C ($1.0\,mg\,kg^{-1}$, i.v.) at a dose near to its i.v. ED_{50} for antitussive activity, did not cause significant depression of ventilation. In addition, at $10\,mg\,kg^{-1}$, i.v. (approximately 10 times i.v. antitussive ED_{50}) there was also no significant depression of ventilation. There was, however, at this dose significant reduction in respiratory rate, but compensation for this in the form of a significant increase in tidal volume was sufficient to offset it. Therefore, there appears to be little or no penetration of BW443C (at doses equal to and 10 times the i.v. antitussive ED_{50}) into the CNS sufficient to result in significant depression of ventilation compared to morphine and codeine. Only after 30 and $60\,mg\,kg^{-1}$, i.v., did BW443C cause significant depression of ventilation (as measured by a % decrease in minute volume). This was due predominantly to decreases in respiratory rate, which again was offset to some extent by increases in tidal volume although not quite sufficient to prevent depression of ventilation.

The ability of naloxone to prevent the depression of ventilation caused by morphine and BW443C in this model indicates that the depression of ventilation is mediated via an opioid receptor mechanism.

In conclusion, these results in the guinea-pig support the hypothesis that the antitussive action of the opiates codeine and morphine and the opioid pentapeptide BW443C are mediated peripherally and therefore do not require penetration of these drugs into the CNS.

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