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Respiratory and cardiovascular effects of the μ-opioid receptor agonist [Lys^7]dermorphin in awake rats

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1 Changes in respiratory variables, arterial blood pressure and heart rate were studied in awake rats after injection of the opioid peptide [Lys^7]dermorphin and its main metabolites, [1-5]dermorphin and [1-4]dermorphin.

2 Fifteen minutes after injection, doses of [Lys^7]dermorphin producing antinociception (i.c.v., 36–120 nmol; s.c., 0.12–4.7 μmol kg^{-1}) significantly increased respiratory frequency and minute volume of rats breathing air or hypoxic inspirates. This respiratory stimulation was reversed to depression by the 5-HT receptor antagonist ritanserin (2 mg kg^{-1}, s.c.), was blocked by naloxone (0.1 mg kg^{-1}, s.c.), significantly reduced by the μ_1 opioid receptor antagonist naloxonazine (10 mg kg^{-1}, s.c., 24 h before) but unaffected by peripherally acting opioid antagonist naloxone methyl bromide (3 mg kg^{-1}, s.c.). Forty five minutes after injection, doses of the peptide producing catalepsy (s.c., 8.3–14.2 μmol kg^{-1}, i.c.v., 360 nmol) significantly reduced respiratory frequency and volume of rats breathing air and blocked the hypercapnic ventilator response of rats breathing from 4% to 10% CO_2. I.c.v. administration of [1-5]dermorphin and [1-4]dermorphin (from 36 to 360 nmol) never stimulated respiration but significantly reduced basal and CO_2-stimulated ventilation. Opioid respiratory depression was only antagonized by naloxone.

3 In awake rats, [Lys^7]dermorphin (0.1–1 mg kg^{-1}, s.c.) decreased blood pressure. This hypotensive response was abolished by naloxone, reduced by naloxone methyl bromide and unaffected by naloxonazine.

4 In conclusion, the present study indicates that analgesic doses of [Lys^7]dermorphin stimulate respiration by activating central μ_1 opioid receptors and this respiratory stimulation involves a forebrain 5-hydroxytryptaminergic excitatory pathway.

Keywords: [Lys^7]dermorphin; opioids; awake rats; respiratory stimulation; respiratory depression; blood pressure

Introduction

Since respiratory depression after therapeutic doses of opioids is a problem of clinical relevance, there is considerable interest in the development of potent analgesics devoid of respiratory depressant side-effects. Numerous studies in which opioid drugs have been compared with morphine in man have found that, when equianalgesic doses are used, the degree of respiratory depression observed was not significantly different from that seen with morphine (see Reisine & Pasternak, 1996). However, studies involving systemic administration of opioids in unanaesthetized animals have to contend with the major problem of species differences in the respiratory effects. While in the monkey, dog, rat and mouse, systemically administered opioids depress breathing, in other species, notably the cat, goat, sheep and cow, they induce respiratory stimulation (Santiago & Normam, 1985). Furthermore, general anaesthesia converts the response of opiate-excited species into one of respiratory depression (see Santiago & Norman, 1985). In rat and mouse, evidence exists that morphine induces respiratory depression by acting on a subpopulation of μ receptors (μ_2) distinct from those involved in the production of supraspinal analgesia (μ_1) (Ling *et al*., 1985). More recently, studies have revealed that some opioid peptides, those that preferentially bind to μ_1 opioid receptors, when injected into lateral ventricles of the brain, stimulate respiration in unanaesthetized rats (Paakkari *et al*., 1993). As these peptides penetrate the blood-brain barrier poorly, respiratory stimulation has not been observed after their systemic administration (Paakkari *et al*., 1993).

Previous work has demonstrated that the opioid peptide [Lys^7]dermorphin has an affinity and selectivity for μ-opioid receptors ten times higher than the μ-opioid agonist [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO) and preferentially activates the μ_1-opioid receptor subtype (Negri *et al*., 1992). When injected subcutaneously this peptide crosses the blood-brain barrier and reduces the nociceptive response to radiant heat, hot water or mechanical pressure with a potency 25–30 times higher than that of morphine (Negri *et al*., 1995). As a preferential μ_1-opioid agonist, [Lys^7]dermorphin would be expected to stimulate respiration or at least to depress it to a lesser extent than morphine. Therefore, the primary purpose of the present work was to investigate the respiratory effects of [Lys^7]dermorphin in awake rats and to compare these effects with those of morphine with respect to the ratio of analgesic to respiratory-depressant activities. However, Negri *et al*. (1992) demonstrated that [Lys^7]dermorphin, although at slow rate, is converted by rat brain enzymes to the N-terminal penta- and tetra-peptides, the last representing the minimal effective structure required to elicit the classical opioid responses (see Melchiorri & Negri, 1996). These metabolic fragments, which are also generated in the periphery, could contribute to the production of some effects observed after [Lys^7]dermorphin administration and thus their effect on ventilation was also evaluated. A secondary target of our experiments was to investigate the site of action of [Lys^7]dermorphin, the receptor subtypes involved in the changes in ventilation and the mechanism by which the respiratory responses are produced.

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The sites of action and the subtype of μ receptors involved in opioid respiratory effects were studied in rats pretreated with the centrally acting μ_1-selective antagonist naloxonazine and the peripherally acting antagonist naloxone methyl bromide.

The mechanism by which opioids depress ventilation is generally considered to be by decreasing the responsiveness of the brainstem respiratory centres to carbon dioxide (see Martin, 1983). However, peripheral chemoreceptor input may still be effective in stimulating respiration, as respiration can be stimulated by hypoxia and inhibited by the inhalation of pure oxygen when opioids have decreased the responsiveness to CO_2 (see Reisine & Pasternak, 1996). Thus, to evaluate the respiratory responses to central and peripheral chemoreceptor input, in the present experiments measurements were made of respiratory variables in rats receiving [Lys^7]dermorphin, breathing inspirates containing different concentrations of oxygen and carbon dioxide. In comparison, the mechanism by which opioids stimulate respiration is poorly understood. For instance, in species in which peripherally injected opioids stimulate breathing, iontophoretic application of these opioids to respiratory neurones in the brain stem depresses their activity (see Bianchi *et al*., 1995). This apparently contradictory effect is most likely to be due to the modulatory role of descending excitatory pathways that stimulate the brain stem respiratory network and/or the phrenic motor nucleus (see Bonham, 1995). At least three different types of excitatory inputs have been identified that modulate the respiratory neuronal network (see Bianchi *et al*., 1995). The first is glutamatergic and is suppressed by opioids. The second is mediated by substance P (SP), and is still effective even when opioids have depressed the respiratory network. A third excitatory input is transmitted by 5-hydroxytryptaminergic projections which originate in the forebrain or raphe nuclei and activate brainstem respiratory neurones and spinal phrenic motoneurones (Davies *et al*., 1988; Lindsay & Feldman, 1993; Anderson *et al*., 1995). Therefore in the present experiments, by using selective substance P (SP) and 5-hydroxytryptamine (5-HT) receptor antagonists, the mechanism by which the opioid stimulant effect is produced in the rat has also been investigated.

Drug-induced changes in cardiovascular function can also affect respiration (see Daly, 1986). Opioids may induce hypertensive or hypotensive responses as well as tachycardic or bradycardic effects, depending largely on dose, route of administration, site of injection, anaesthesia and stress (Evans *et al*., 1952; Lemaire *et al*., 1978; Calignano *et al*., 1992; Randich *et al*., 1993). To correlate cardiovascular changes with pulmonary ventilatory changes, arterial blood pressure and heart rate were monitored in awake rats receiving [Lys^7]dermorphin or morphine, with and without pretreatment with μ-opioid receptor antagonists.

Methods

Animals

Male Wistar rats (220–250 g) were used for the experiments. They were housed at a temperature of $22 \pm 2^{\circ}$C, with food and water *ad libitum*. A standard light/dark cycle was maintained with a time-regulated light period from 06 h to 18 h. All animal studies were conducted in accordance to the Italian Law for Care and Use of Laboratory Animals and the research project was accredited by the Ministry of the University and Scientific and Technological Research (MURST, Italy). A total of 844 rats was used, each rat receiving one dose of one drug or one agonist-antagonist combination. Rats were divided into eleven groups, including vehicle injected controls. A group of 256 rats was used to study respiratory changes produced by s.c. (160 rats) and i.c.v. (96 rats) injection of [Lys^7]dermorphin. A group of 210 rats was used in experiments in which receptor antagonists were tested for preventing [Lys^7]dermorphin produced respiratory changes. A group of 16 rats was exposed twice to increasing concentrations of CO_2, the first time after s.c. injection of vehicle and the second time after s.c. injection of [Lys^7]dermorphin alone (8 rats) or [Lys^7]dermorphin combined with ritanserin (8 rats). A group of 25 rats was used to calculate the dose-response curve for [Lys^7]dermorphin-induced antinociception. A group of 33 rats was used to measure arterial blood pressure after [Lys^7]dermorphin alone (20 rats) or after [Lys^7]dermorphin combined with opioid antagonists (13 rats). A group of 72 rats received s.c. doses of morphine to study respiratory changes. A group of 8 rats was used to obtain the CO_2 dose-response curve in the presence of morphine and a group of 36 rats received opioid receptor antagonists before morphine. A group of 20 rats were used to calculate the dose-response curve for morphine-induced antinociception and 8 rats receiving s.c. morphine were used to measure arterial blood pressure changes. Finally, 160 rats were used to measure respiratory changes induced by N-terminal fragments of [Lys^7]dermorphin.

Recording of respiratory and cardiovascular variables

Ventilation rate, tidal volume and minute volume were measured by means of the whole body plethysmograph method described by Bartlett and Tenney (1970). A Validyne MP45-871 differential pressure transducer (Validyne Corp., Northridge, CA, USA) with a pressure range ± 2 cmH_2O was connected to the animal and compensatory chamber. The two chambers were made of plexiglass and were identical in size ($145 \times 205 \times 234$ mm, 7 l) and construction. Lids had hermetic closures and inlet and outlet tubes were connected to the chambers to ventilate them with humidified room air or with test gases at a flow rate of 7 l min^{-1}. A Validyne carrier demodulator was used for the excitation, amplification, and filtering necessary to produce a d.c. output signal proportional to the differential pressure sensed by the transducer. The output signal from the demodulator was conveyed to a bridge amplifier (BM 614/2, Biomedica Mangoni, Pisa, Italy), and then digitized by a 16 bit analogue to digital converter card (ISC-16 card, R.C. Electronics, Goieta, CA, U.S.A.), operating on a 386 INTEL personal computer. Data acquisition and calculations were performed by use of pulmonary-respiratory analysis software obtained from R.C. Electronics (Goieta, CA, U.S.A.). The inlet- and outlet-tubes of the chambers were opened and closed by computer-operated electromagnetic valves to flush the chamber with gas mixtures or to interrupt gas glow during the 1 min-recording of respiratory activity. Tidal volume (V_t), minute volume (V_e) and respiratory frequency (f) were calculated from the equation derived by Epstein and Epstein (1978). Minute volume was expressed as ml min^{-1} kg^{-1}, tidal volume as ml kg^{-1}, and respiratory frequency as breaths min^{-1}.

Tail arterial blood pressure was measured by the non invasive Riva Rocci method in awake restrained rats maintained at an ambient temperature of 30°C. Blood pressure and heart rate (HR) were measured with a BP electronic recorder 8006 (Ugo Basile, Italy). The method involved the monitoring of the pulse on the tail while slowly inflating a cuff that occludes blood flow to the tail. The cuff pressure that stops the pulse was taken as the systolic blood pressure value.

Rats were warmed to 30°C to increase blood flow to the tail for better pulse reading.

Test of antinociception

Nociceptive responses were evoked in rats by exposing the tail to radiant heat (D'Amour & Smith, 1941). The latency of the first sign of a rapid tail-flick was taken as the end point. Before drug administration, rats not flicking their tails within 5 s were eliminated from the study. After drug injection, animals not flicking their tails within 15 s were removed from the nociceptive stimulus and assigned a maximal antinociceptive response. The antinociceptive response was tested 60 min after morphine and 90 min after [Lys^7]dermorphin and calculated as previously described (Negri *et al.*, 1995). Five rats were used for each opioid dose.

Intracerebroventricular and intravenous injections

Under ketamine-xylazine anaesthesia (90 mg kg^{-1} + 10 mg kg^{-1}, i.p.) a plastic guide cannula was implanted in the skull over the left lateral ventricle, by drilling a hole 1.5–2 mm lateral and posterior to the junction of the sagittal and coronal sutures. After one week recovery from surgery, awake rats were intracerebroventricularly (i.c.v.) injected with a Hamilton microsyringe connected with a short plastic tube (PE 10) to a 27 G needle inserted in the cerebral cannula at a depth suitable to reach the lateral ventricle. No more than 5 μl of solution was slowly injected into the ventricle at a rate of 0.1 μl s^{-1} and the needle was left in place for 10 s after the end of the injection to avoid reflux.

Intravenous injections were made into a tail vein after immersion of the tail in hot water (40°C) for 1–2 min to dilate the veins. From 0.1 to 0.2 ml of drug solution was injected.

Experimental protocol

To record respiratory variables rats were transferred to the animal chamber of the plethysmograph and accustomed to the environment for a period of 60 min during which time both chambers were flushed with humidified room air. After a 30–40 min exploratory period rats appeared calm and remained quiet throughout the experimental session. After the 60 min adaptation period, respiratory tests began. Each respiratory test consisted of 4 steps: 1 min recording of respiratory activity in air with the inlet and outlet tubes of the chambers closed; 4 min flushing of both chambers with humidified test gases; 1 min recording of respiratory activity with the inlet and outlet valves of the chambers closed; and final flushing of the chambers with humidified room air. The time-course of respiratory activity was recorded over a 300 min observation period by use of the following procedure. The respiratory test was repeated every 15 min during a 1 h basal period, after which the animal chamber was opened and the rat was injected with the drug or vehicle. During the following two hours the respiratory test was repeated every 15 min and from the 3rd h onwards every 30 min. In each rat, respiratory variables were analysed and averaged during steady-state ventilation in the 30 s before and during the last 30 s of exposure to room air or one of the following test gases: 100% O_2 (hyperoxia); 10% O_2, 90% N_2, (hypoxia); 7% CO_2, 20% O_2, 73% N_2 (normoxic hypercapnia); 7% CO_2, 93% O_2, (hyperoxic hypercapnia). For the same rat, breathing air and one of the test gases, ventilation variables were determined on the first day under saline and on the second day under one dose of the test drug. Each rat received a drug only once and eight rats were used for each drug dose and for each test gas. For the CO_2 response curve, chambers were flushed with increasing concentrations of CO_2. The respiratory test lasted 21 min and consisted of the following steps: 1 min recording from air breathing rat; 4 min flushing of the chambers with 2% CO_2, 20% O_2, 78% N_2; 1 min recording; 4 min flushing of the chambers with 4% CO_2, 20% O_2, 76% N_2; 1 min recording; 4 min flushing of the chambers with 7% CO_2, 20% O_2, 73% N_2; 1 min recording; 4 min flushing of the chambers with 10% CO_2, 20% O_2, 70% N_2; 1 min recording followed by air flushing of the chambers. For each rat the test was made on the first day under saline and on the second day under [Lys^7]dermorphin or morphine. Eight rats were used for each opioid, each rat receiving one drug and only one dose of that drug. The test began 80 min after s.c. injection of [Lys^7]dermorphin and 50 min after morphine.

In experiments in which receptor antagonists were tested, two groups of rats, equal in number of animals (4), were used for each gas mixture, one group receiving the antagonist alone and the other the antagonist followed by the test opioid.

To measure changes in systolic blood pressure, rats with cuffs and transducers in place were acclimatized to a warming chamber (30°C) for 3 h before testing. Without removing the rats from the chamber, a first pressure record was taken before saline injection. After s.c. saline injection three blood pressure measurements were performed five minutes apart. Rats were then injected with the drug and blood pressure measured every 5 min during the first 40 min after drug injection. From the 40th min onwards, blood pressure was measured every 20 min over a period of 3 h. Each rat received one drug and one dose of that drug. Four rats were used for each drug dose.

Data analysis

Data obtained from experiments on the time-course of respiratory activity were collected and analysed as follows. Before saline or drug injection, ventilation variables of each rat breathing air and one gas mixture were measured three times at 15 min intervals, averaged and referred to as baseline values for that rat and inspirate. Ventilator variables were again measured during four post-injection periods: from 15 to 45 min, 60 to 105 min, 120 to 180 min, and 240 to 300 min after saline or drug injection. For each rat and each post-injection period, the maximum change in each ventilator variable, from the baseline value, was calculated. Changes produced by the same treatment and occurring in the same post-injection period were averaged to give the mean maximum change of the ventilatory variable. For each post-injection period, the mean maximum change produced by each dose of test opioid was compared to that produced by saline in the same group breathing the same inspirate. The changes in ventilator variables are expressed as means ± s.e.mean. In figures in which absolute values of ventilatory variables are presented, mean ventilatory values recorded at each time point after opioid injection were compared with pre-injection values. Blood pressure changes produced by drugs were compared with those produced by saline in the same animals. Statistical analysis was made by two-way ANOVA followed by Newman-Keuls test (see Winer, 1971), an *a posteriori* test to compare means.

Drug treatment and drugs

[Lys^7]dermorphin was injected s.c. at dose levels of 0.12, 1.18, 4.73, 8.3 and 14.2 μmol kg^{-1} (0.1, 1.0, 4.0, 7.0 and 12.0 mg kg^{-1}) and i.c.v. at dose levels of 36, 120, 360 nmol (30, 100, 300 ng). The N-terminal fragments of [Lys^7]dermor-

phin were injected i.c.v. at the same molar doses as [Lys^7]dermorphin and s.c. at doses of 1, 5, 20 and 40 μmol kg^{-1}. Morphine HCl was injected s.c. at the doses of 3.3, 6.7, 13.3, 26.6, 53.2 and 106.4 μmol kg^{-1} (1.25, 2.5, 5.0, 10.0, 20.0 and 40.0 mg kg^{-1}). Naloxonazine dihydrate was injected i.v. at a dose of 10 mg kg^{-1}, 24 h before testing opioids. Naloxone HCl (0.1 mg kg^{-1}, s.c.) and naloxone methyl bromide (3 mg kg^{-1}, s.c.) were injected 15 min before the opioid being tested. Metergoline phenylmethyl ester (1 mg kg^{-1}, s.c.), a non selective 5-HT$_1$/5-HT$_2$/5-HT$_7$ antagonist (see Peroutka, 1993; Jasper *et al*., 1997), WAY-100635 (0.3 mg kg^{-1}, s.c.), a 5-HT$_{1A}$ receptor antagonist (Khawaja, 1995; Khawaja *et al*., 1995; Forster *et al*., 1995), GR127935 (1 mg kg^{-1}, i.v.), a 5-HT$_{1B/1D}$ receptor antagonist (Skingle *et al*., 1996), ritanserin (2 mg kg^{-1}, s.c.), a 5-HT$_{1D}$/5-HT$_2$/5-HT$_7$ receptor antagonist (Peroutka, 1993; Jasper *et al*., 1997), and prazosin hydrochloride (2 mg kg^{-1}, s.c.), an α_1-adrenoceptor antagonist, were injected 30 min before opioid injection. SR140333, an antagonist at tachykinin NK$_1$ receptors (Emonds-Alt *et al*., 1993), and SR48968, an antagonist at tachykinin NK$_2$ receptors (Croci *et al*., 1995; Santucci *et al*., 1993), were injected i.c.v. at the doses of 1 and 3 μg per rat, at the time of opioid injection. For i.c.v. and s.c. injections, [Lys^7]dermorphin and its N-terminal fragments were dissolved in saline (0.9% NaCl). To dissolve prazosin and metergoline, 10 mg of each drug was solubilized in 1 ml of 45% w/v 2-hydroxypropyl-β-cyclodextrin in H_2O and the solution lyophilized to form a fully soluble powder (Pitha *et al*., 1988). The powder (460 mg) was then dissolved in 30 ml of saline and injected in a volume of 1–6 ml kg^{-1} (s.c.). Ritanserin was dissolved in a saline-Tween 80 2% solution to a strength of 0.5 mg ml^{-1}, immediately before s.c. injection. Control rats were injected with the coresponding vehicle solutions (15 mg ml^{-1} of 2-hydroxypropyl-β-cyclodextrin or Tween 80 2% in saline). When expressed as mg kg^{-1}, doses refer to the salts of the drug.

Morphine HCl and naloxone HCl were purchased from SALARS (Como, Italy). Naloxonazine, naloxone methyl bromide and ritanserin were obtained from RBI (Natick, MA, U.S.A.), metergoline phenylmethyl ester, prazosin hydrochloride and 2-hydroxypropyl-β-cyclodextrin were purchased from Tocris Cookson Ltd (Bristol, U.K.). WAY-100635 (N-[2-[4-(2-methoxphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclo-hexanecarboxamide 3HCI) and GR127935 (2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide HCl H_2O), were obtained from Wyeth S.p.a. (Milano, Italy) and Glaxo-Wellcome S.p.A. (Milano, Italy), respectively. [Lys^7]dermorphin and its N-terminal fragments Tyr-D-Ala-Phe-Gly-NH$_2$ ([1-4]dermorphin) and Tyr-D-Ala-Phe-Gly-Tyr-NH$_2$ ([1-5]dermorphin) were synthesized as previously described (Negri *et al*., 1992). SR140333 ((**S**)1-(2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxy-phenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo[2.2.2]octane chloride) and SR48968 ((S)-N-methyl-N-[4-4-acetylamino-4-phenylpiperidine)-2-(3,4-dichlorophenyl)butyl]benzamide) were gift from G. Le Fur and X. Emonds-Alt (SANOFI, Montpellier, France).

Results

Respiratory responses of the conscious unrestrained rat

Baseline values of ventilatory variables measured in rats breathing air or hypercapnic, hyperoxic and hypoxic inspirates corresponded reasonably well with those determined previously in the unrestrained rat by means of whole body plethysmography (Bartlett & Tenney, 1970; Olson & Dempsey, 1978; Lai *et al*., 1981; Maskrey *et al*., 1981). By averaging the baseline values measured in all rat groups before s.c. and i.c.v. saline injection the following mean values (±s.e.mean) were obtained: for air inspirates, minute volume (V_e) 595.7±39.2 ml min^{-1} kg^{-1}, tidal volume (V_t) 6.07±0.74 ml kg^{-1} and respiratory frequency (f) 98.3±8.2 breath min^{-1}; for inspirates containing 7% CO_2, 21% O_2, 72% N_2, V_e=1475±109 ml min^{-1} kg^{-1}, V_t=11.4±0.8 ml kg^{-1} and f=120.3±9.4 breath min^{-1}; for 100% O_2 inspirates, V_e=606±46 ml min^{-1} kg^{-1}, V_t=6.5±0.5 ml kg^{-1} and f=96.7±6.4 breath min^{-1}; for inspirates containing 7% CO_2, 93% O_2, V_e=1166±107 ml min^{-1} kg^{-1}, V_t=9.9±0.7 ml kg^{-1} and f=112.3±7.39 breath min^{-1}; for 10% O_2 inspirates, V_e=1087±86 ml min^{-1} kg^{-1}, V_t=7.4±0.6 ml kg^{-1} and f=141.7±11.5 breath min^{-1}. Figure 1 illustrates an example of spirogram recording from a rat breathing air, hypercapnic inspirates (7% CO_2, 21% O_2, 72% N_2) and hypoxic inspirates (10% O_2, 90% N_2). Hypercapnic inspirates produced a sharp increase in the amplitude of pressure waves that are associated with each tital breath. Hypoxia increased respiratory frequency more than tidal volume.

I.c.v. injection of 5 μl of saline or s.c. injection of 1 ml of saline did not produce significant changes in ventilatory responses to gas mixtures during the four observation periods. Mean ΔV_e values were within ± 50 ml min^{-1} kg^{-1}, i.e. within ±10% baseline value recorded before saline injection (data not shown).

Respiratory responses to opioids

Respiratory stimulation In rats breathing air or hypoxic gas mixture, [Lys^7]dermorphin dose-dependently stimulated respiratory frequency (Figure 2a). The increase in respiratory frequency was already evident 15 min after s.c. injection of 1.2 μmol kg^{-1} of the peptide (Δf=18.7±3.2 breath min^{-1}, $P<0.05$), reached a maximum (Δf=54.8±8.1 breath min^{-1}, $P<0.001$) 30 min after 14.2 μmol kg^{-1} dose and lasted 45–50 min. In rats breathing hypoxic inspirates tidal volume significantly increased only after 0.12 and 1.2 μmol kg^{-1} dose

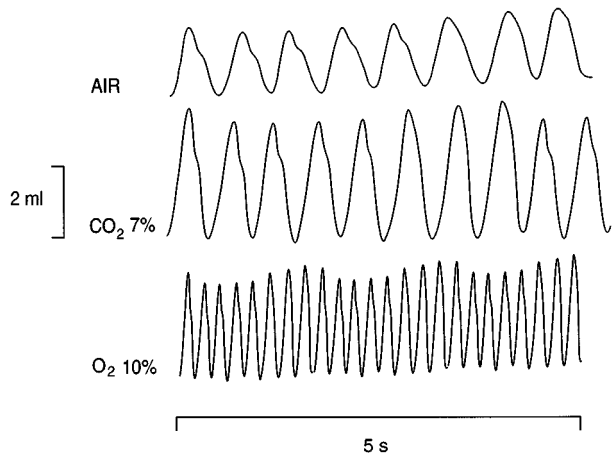

Figure 1 Spirogram recording from a conscious rat breathing air, hypercapnic inspirates (7% CO_2, 21% O_2, 72% N_2) and hypoxic inspirates (10% O_2, 90% N_2). Respiratory activity was registered for 5 s with the whole body plethysmograph method described by Bartlett and Tenney (1970). The height of each pressure wave measures the tidal volume of each breath. The calibration bar on the left indicates a wave height corresponding to a tidal volume of two ml.

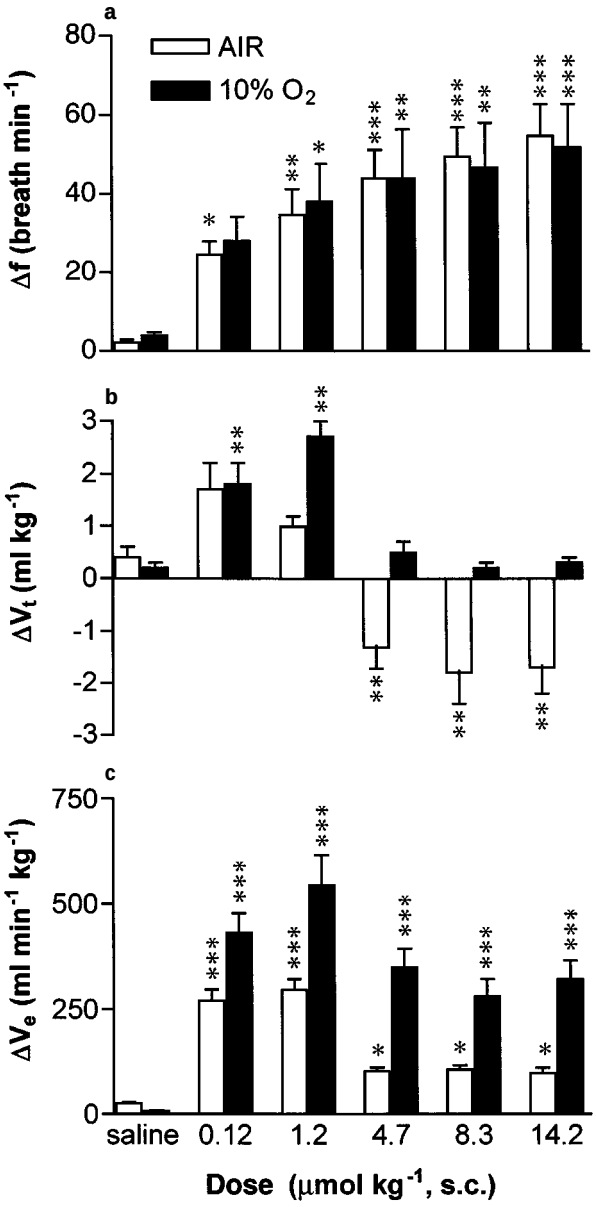

Figure 2 Changes in (a) respiratory frequency (Δf), (b) tidal volume (ΔV_t) and (c) minute volume (ΔV_e) from baseline values produced by graded s.c. doses of [Lys^7]dermorphin in conscious rats breathing air or hypoxic inspirates (10% O_2, 90% N_2). Each column represents the mean of maximum changes occurring in 8 rats within 45 min from peptide injection. Vertical lines show s.e.mean. Newman-Keuls multiple comparison test: $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to saline.

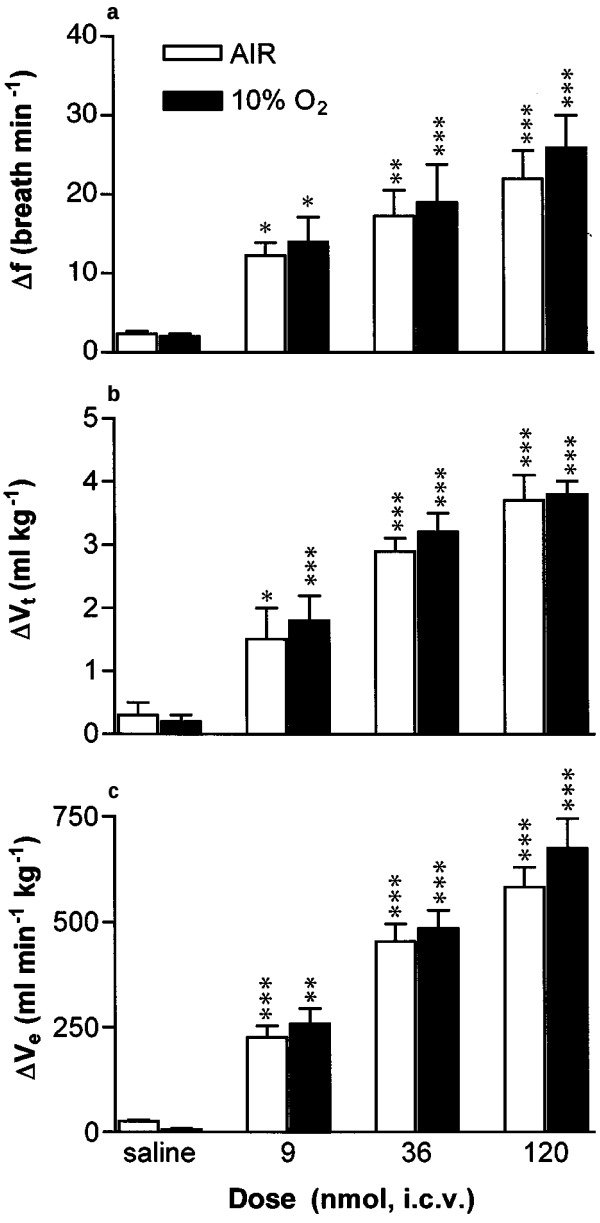

Figure 3 Changes in (a) respiratory frequency (Δf), (b) tidal volume (ΔV_t) and (c) minute volume (ΔV_e) from baseline values produced by graded i.c.v. doses of [Lys^7]dermorphin in conscious rats breathing air or hypoxic inspirates (10% O_2, 90% N_2). Each column represents the mean of maximum changes occurring in 8 rats within 45 min from peptide injection. Vertical lines show s.e.mean. Newman-Keuls multiple comparison test: $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to saline.

($\Delta V_t = 0.98 \pm 0.21$ ml kg^{-1} at 0.12 μmol kg^{-1} dose) while it decreased in air breathing rats after higher doses of the peptide, reaching a minimum ($\Delta V_t = -1.8 \pm 0.5$ ml kg^{-1}, $P<0.01$) 30 min after the 8.3 μmol kg^{-1} dose (Figure 2b). As a consequence of these opposite changes in respiratory frequency and tidal volume, the increase in minute volume did not correlate with the peptide dose (Figure 2c).

After i.c.v. injection and up to the 120 nmol dose, [Lys^7]dermorphin dose-dependently increased both normoxic and hypoxic ventilation of freely moving rats (Figure 3). After i.c.v. injection of the 120 nmol dose, this increase in pulmonary ventilation reached a maximum of 583 ± 47 ml min^{-1} kg^{-1} ($P<0.001$) in air breathing rats and of 675 ± 70 ml min^{-1} kg^{-1} ($P<0.001$) in hypoxic rats and was due to an increase in tidal volume and respiratory frequency. The respiratory stimulation peaked 15 min after [Lys^7]dermorphin injection and lasted 30 min.

Neither s.c. nor i.c.v. doses of [1-5]dermorphin, [1-4]dermorphin or morphine increased ventilation of rats breathing air or hypoxic inspirates (data not shown). Naloxone (0.1 mg kg^{-1}, s.c.), blocked and naloxonazine (10 mg kg^{-1}, i.v., 24 h before) significantly and potently reduced the respiratory stimulation produced by [Lys^7]dermorphin (1.2 μmol kg^{-1}, s.c.), but naloxone methyl bromide (3 mg kg^{-1}, s.c.) did not (Figure 4a).

In preliminary experiments, metergoline (1 mg kg^{-1}, s.c.) did not modify baseline respiratory variables but significantly reduced the respiratory stimulation produced by [Lys^7]der-

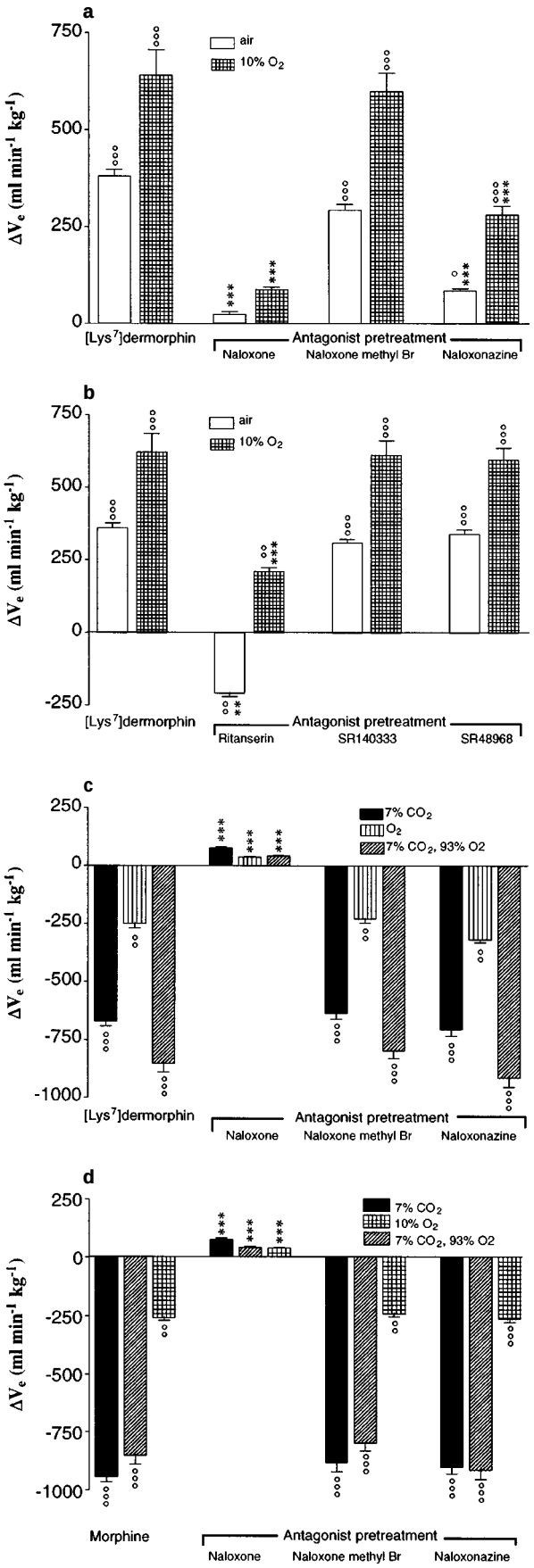

Figure 4 Effects of opioid antagonists (naloxone, naloxone methyl bromide, naloxonazine), 5-HT receptor antagonist ritanserin, and NK_1- and NK_2-receptor antagonists (SR140333 and SR49968) on respiratory changes produced by s.c. administration of [Lys⁷]dermorphin and morphine in conscious rats. (a) and (b), Effects of antagonists on the maximum increase in respiratory volume (ΔV_e) produced by 1.2 μmol kg^{-1} [Lys⁷]dermorphin in rats breathing air or 10% oxygen. (c) Effects of opioid antagonists on the maximum decrease in respiratory volume produced by 14.2 μmol kg^{-1} [Lys⁷]dermorphin in rats breathing 7% carbon dioxide, pure oxygen or 7% carbon dioxide in oxygen. (d) Effects of opioid antagonists on respiratory depression produced by 13.3 μmol kg^{-1} morphine in rats breathing 7% carbon dioxide, 10% oxygen or 7% carbon dioxide in oxygen. Data are expressed as the mean of maximum changes occurring in 4 rats. Vertical lines show s.e.mean. Newman-Keuls multiple comparison test: $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$ and $^{\circ\circ\circ}P<0.001$ *vs* saline injected rats; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* opioid injected rats.

morphin (1.2 μmol kg^{-1}, s.c.) in air breathing rats (data not shown). Among the 5-HT antagonists tested, WAY-100635 (0.3 mg kg^{-1}, s.c.) and GR127935 (1 mg kg^{-1}, i.v.) failed to modify the baseline respiratory variables and the respiratory response to the opioid, but ritanserin significantly and potently reduced [Lys⁷]dermorphin-stimulated hypoxic ventilation and converted the normoxic ventilatory stimulation into ventilatory depression (Figure 4b). In contrast, the α_1-adrenoceptor antagonist prazosin, the NK_1- and the NK_2-antagonists SR140333 and SR48968 were ineffective.

Respiratory depression In air-breathing rats, during the second hour of the study period, s.c. administration of 8.3 and 14.2 μmol kg^{-1} [Lys⁷]dermorphin significantly lowered respiratory frequency with a simultaneous compensatory increase in tidal volume and without any significant change in minute volume. For the 14.2 μmol kg^{-1} dose the following maximum changes in the ventilatory variables occurred 90–105 min after s.c. injection of the opioid: $\Delta f = -44 \pm 8$ breath min^{-1}, $P<0.001$; $\Delta V_t = +3.2 \pm 1.4$ ml min^{-1}, $P<0.01$; and $\Delta V_e = -62 \pm 11$ ml^{-1} kg^{-1}, $P>0.05$. By the same route of administration, the dose of 40 μmol kg^{-1} [1-5]dermorphin halved respiratory frequency ($\Delta f = -42.8 \pm 6$ breath min^{-1}, $P<0.01$), slightly increased tidal volume ($\Delta V_t = +1.7 \pm 0.6$ ml min^{-1}, $P<0.05$) and significantly decreased minute volume ($\Delta V_e = -243 \pm 37$ ml min^{-1} kg^{-1}, $P<0.05$). This minute volume depression was four times more intense than that produced by 14.2 μmol kg^{-1} [Lys⁷]dermorphin ($P<0.05$) and its nadir occurred 45 min after s.c. injection.

Subcutaneous doses of morphine up to 6.7 μmol kg^{-1} had no consistent effect on respiratory variables but 13.3 μmol kg^{-1} of the alkaloid significantly decreased both tidal volume ($\Delta V_t = -2.3 \pm 0.5$ ml min^{-1}, $P<0.01$) and minute volume ($\Delta V_e = -205 \pm 21$ ml min^{-1} kg^{-1}, $P<0.05$) and significantly increased respiratory frequency ($\Delta f = 24 \pm 8$ breath min^{-1}, $P<0.05$). The nadir of morphine-induced ventilator depression was reached 60 min after the 53.2 μmol kg^{-1} dose, when ventilation declined ($\Delta V_e = -357 \pm 42$ ml min^{-1} kg^{-1}, $P<0.001$) and respiratory frequency rose ($\Delta f = 25 \pm 7$ breath min^{-1}, $P<0.05$). Thus morphine depressed ventilation of air-breathing rats mainly through a fall in tidal volume.

From the 30th min after i.c.v. injection onwards, the 360 nmol dose of [Lys⁷]dermorphin significantly and progressively lowered respiratory frequency and decreased tidal volume and minute volume of air breathing rats. The depression of ventilation peaked at 60 min ($\Delta V_e = -255 \pm 36$ ml min^{-1} kg^{-1}, $P<0.05$) and lasted about 60 min (Figure 5b). The N-terminal fragments of [Lys⁷]dermorphin acted four times faster than the parent compound since 15 min after i.c.v. injection of 120 nmol [1-5]dermorphin both tidal volume and respiratory frequency were significantly reduced to

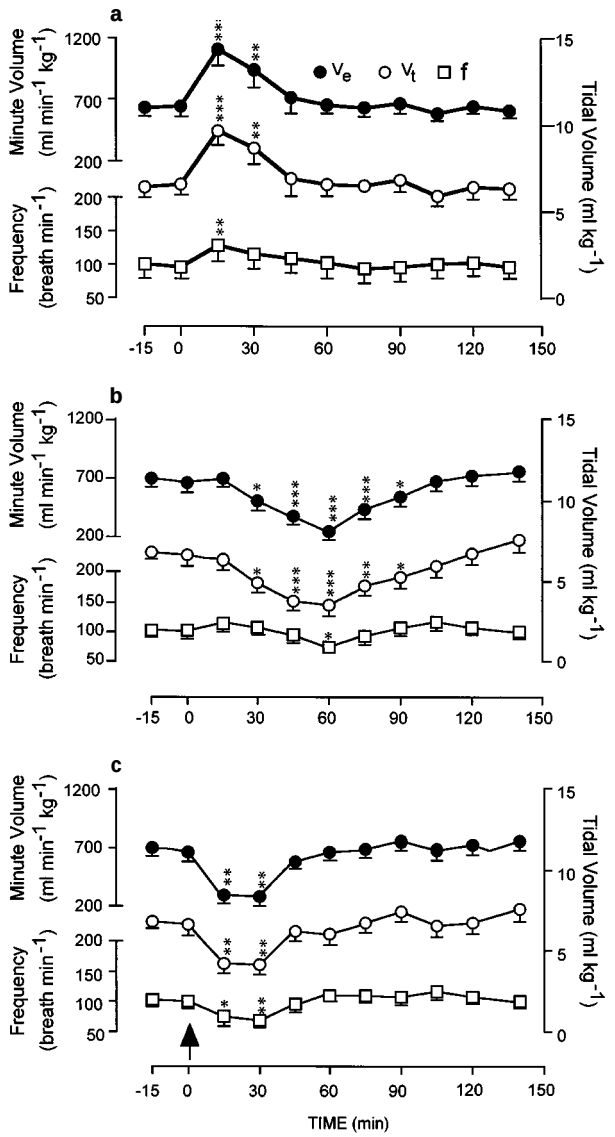

Figure 5 Time-response curves of respiratory frequency (*f*), tidal volume (V_t) and minute volume (V_e) in conscious rats breathing air after i.c.v. injection of: (a) 36 nmol of [Lys^7]dermorphin; (b) 360 nmol of [Lys^7]dermorphin; (c) 120 nmol of [1-5]dermorphin. Arrow indicates the time point at which peptides were injected. Data are expressed as the mean of respiratory variables of 8 rats. Vertical lines show s.e.mean. Newman-Keuls multiple comparison test: $*P<0.05$, $**P<0.01$ and $***P<0.001$ compared to pre-injection values.

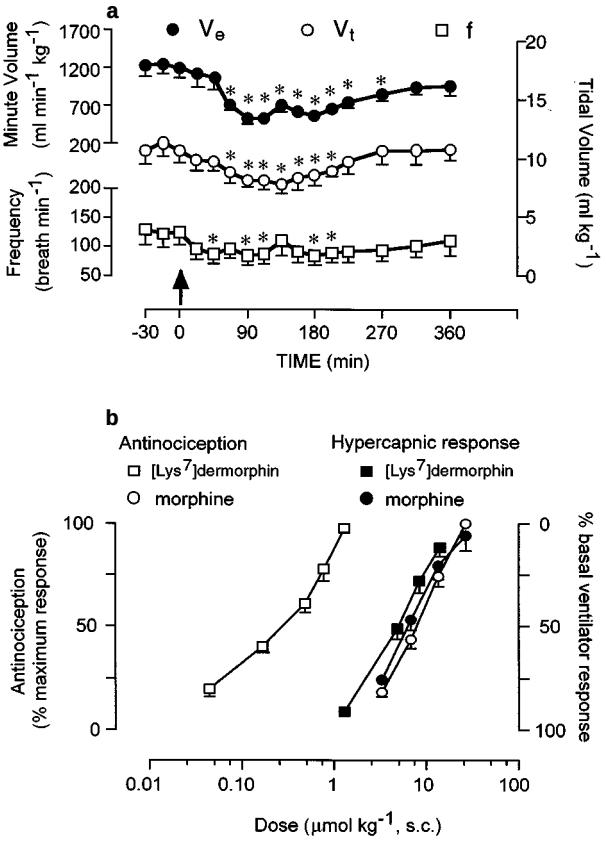

Figure 6 (a) Time-response curves of respiratory frequency (*f*), tidal volume (V_t) and minute volume (V_e) of conscious rats breathing hypercapnic gas mixtures (7% CO_2, 20% O_2, 73% N_2) and injected s.c. with 14.2 μmol kg^{-1} of [Lys^7]dermorphin (arrow). Data are expressed as the mean of respiratory variables of 8 rats. Vertical lines show s.e.mean. Newman-Keuls multiple comparison test: $*P<0.01$ compared with preinjection values. (b) Dose-response curves for antinociception (open symbols, $n=5$) and hypercapnic respiratory depression (solid symbols, $n=8$) produced by [Lys^7]dermorphin and morphine. Each point represents mean and vertical lines show s.e.mean. Changes in the respiratory response to hypercapnia produced by doses of the test opioid were measured 60 min after s.c. morphine or 90 min after s.c. [Lys^7]dermorphin. ED_{50} doses for antinociception and inhibition of the hypercapnic respiratory response, calculated by nonlinear regression analysis of dose-response curves, were: antinociception, 0.33 ± 0.12 μmol kg^{-1} [Lys^7]dermorphin and 7.5 ± 1.7 μmol kg^{-1} morphine; respiratory response, 4.6 ± 0.15 μmol kg^{-1} [Lys^7]dermorphin and 5.9 ± 1.1 μmol kg^{-1} morphine.

approximately 60% of the baseline value, and minute volume lowered to one third the baseline value. This depression of ventilation lasted about 30 min (Figure 5c).

In rats breathing hypercapnic inspirates [Lys^7]dermorphin produced a respiratory depression that began 60 min after s.c. injection of the 14.2 μmol kg^{-1} dose, peaked after 90 min and lasted for at least 5 h (Figure 6a). Since time-response curves demonstrated that maximum depression of the hypercapnic response occurred 45 min after morphine and 90 min after [Lys^7]dermorphin injection, we compared the antinociceptive potency and respiratory depressant activity of [Lys^7]dermorphin with those of morphine at these time points (Figure 6b). For the peptide, the ratio of antinociceptive to respiratory depressant ED_{50} dose was 0.07 while for morphine the ratio was 1.2, i.e. 17 times higher. We further compared the ventilatory responses to equipotent doses of the two opioids in rats breathing a gas mixture the CO_2 content of which was raised stepwise from 2% to 10% (Figure 7). The dose of 4.7 μmol kg^{-1} of the peptide did not affect the 2% CO_2-induced ventilatory response, slightly depressed the response to 4% CO_2 and halved the ventilatory responses to 7% and 10% CO_2. In contrast, 6.7 μmol kg^{-1} morphine depressed the ventilatory response to all CO_2 concentrations.

The depression of the hypercapnic ventilatory response produced by s.c. doses of 14.2 μmol kg^{-1} [Lys^7]dermorphin and 13.3 μmol kg^{-1} morphine was only blocked by naloxone (0.1 mg kg^{-1}, s.c.) (Figure 4c,d). Pretreatment with ritanserin potentiated the respiratory depressant activity of [Lys^7]dermorphin. After ritanserin (2 mg kg^{-1}, s.c.) 4.7 μmol kg^{-1} [Lys^7]dermorphin significantly depressed the ventilatory responses to inspirates containing 2% and 4% CO_2 (Figure 7).

The depression of the hypercapnic ventilatory response after i.c.v. injection of the peptide was also of late onset (45 min) in comparison with the stimulation of normocapnic ventilatory

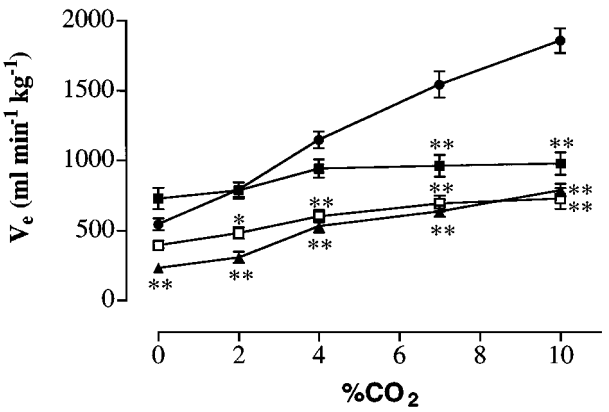

Figure 7 Respiratory minute volume (V_e) of conscious rats breathing graded CO_2 concentrations after s.c. injection of saline, 4.7 μmol kg^{-1} [Lys^7]dermorphin, 6.7 μmol kg^{-1} morphine and 2 mg kg^{-1} ritanserin plus 4.7 μmol kg^{-1} [Lys^7]dermorphin. Data are expressed as the mean of respiratory volumes of 8 rats. Newman-Keuls multiple comparison test: $*P<0.05$; $**P<0.01$ compared with saline.

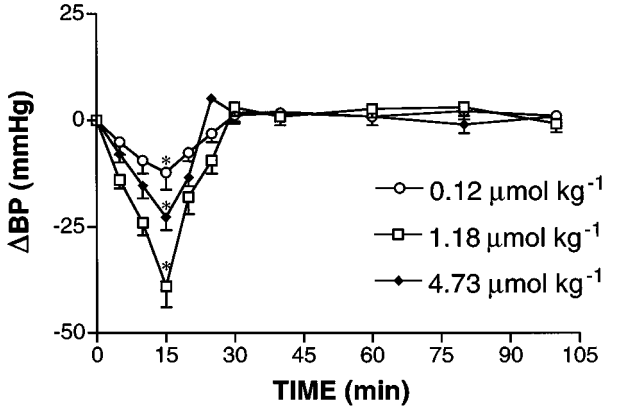

Figure 8 Time-course of systolic arterial blood pressure changes (ΔBP) produced by s.c. injection of [Lys^7]dermorphin in awake rats. Data are expressed as the mean of ΔBP of 4 rats. Peptide was injected s.c. at time 0 min. Blood pressure was measured from rat tail artery (Riva Rocci method). Newman-Keuls multiple comparison test: $*P<0.01$, compared with saline.

response (15 min). Sixty mminutes after i.c.v. injection, the dose of 120 nmol of the peptide halved the hypercapnic ventilatory response ($\Delta V_e = -725 \pm 87$ ml min^{-1} kg^{-1}, $P<0.001$) and the dose of 360 nmol abolished it ($\Delta V_e = -985 \pm 98$ ml min^{-1} kg^{-1}, $P<0.001$). In contrast, after i.c.v. injection of 120 nmol [1-5]dermorphin the ventilatory response to 7% CO_2 fell within 30 min ($\Delta V_e = -680 \pm 78$ ml min^{-1} kg^{-1}, $P<0.001$). Thus the N-terminal pentapeptide of [Lys^7]dermorphin inhibited the hypercapnic response as the parent peptide did, but acted significantly more rapidly. In rats breathing pure oxygen, during the second post-injection hour, a s.c. dose of 14.2 μmol kg^{-1} [Lys^7]dermorphin significantly decreased pulmonary ventilation (Figure 4c). Ventilatory variables returned to baseline values during the third observation period (120–180 min after injection). Morphine, at a dose of 13.3 μmol kg^{-1}, decreased V_t and V_e of oxygen-breathing rats (Figure 4d) with a small compensatory increase in f. Naloxone pretreatment (0.1 mg kg^{-1}, s.c., $n=4$) abolished respiratory depression produced by the two opioids. Pretreatments with naloxonazine (10 mg kg^{-1}, i.v., 24 h before, $n=4$), naloxone methyl bromide (3 mg kg^{-1}, s.c., $n=5$), ritanserin (2 mg kg^{-1}, s.c., $n=5$), SR140333 (1 μg, i.c.v., $n=4$) or SR48968 (3 μg, i.c.v., $n=4$) were ineffective.

The ventilatory response to hyperoxic hypercapnic inspirates (7% CO_2, 93% O_2) was more sensitive to [Lys^7]dermorphin inhibition than the response to normoxic hypercapnia. While in rats breathing a gas mixture containing 7% CO_2, 93% O_2 the baseline ventilatory response ($V_e = 1112 \pm 118$ ml min^{-1} kg^{-1}, $V_t = 9.3 \pm 1.1$ ml kg^{-1} and $f = 118.3 \pm 6.9$ breath min^{-1}) did not significantly differ from that recorded for normoxic hypercapnic inspirates, the ventilatory response after 4.73 μmol kg^{-1} [Lys^7]dermorphin was strongly reduced ($\Delta V_e = -636 \pm 88$). At the dose of 14.2 μmol kg^{-1} of the peptide the ventilatory depression was so serious that two rats out of 8 died (Figure 4c).

In rats breathing hypoxic hypercapnic inspirates morphine-induced ventilatory depressant effects were not significantly different from those observed in normoxic hypercapnia (Figure 4d).

The inhibition of the hypoxic hypercapnic responses produced by [Lys^7]dermorphin (14.2 μmol kg^{-1}, s.c.) and morphine (13.3 μmol kg^{-1}, s.c.) was antagonized by naloxone (0.1 mg kg^{-1}, s.c.), but not by either naloxonazine (10 mg kg^{-1}, i.v., 24 h before) or naloxone methyl bromide (3 mg kg^{-1}, s.c.) (Figure 4c,d). Ritanserin (2 mg kg^{-1}, s.c., $n=4$), prazosin (2 mg kg^{-1}, s.c., $n=4$), SR140333 (1 μg, i.c.v., $n=4$) and SR48968 (3 μg, i.c.v., $n=4$) did not modify the respiratory depression produced by the peptide.

Effects of opioids on arterial blood pressure

Arterial systolic blood pressure and heart rate values of awake restrained rats ($n=33$) were 137 ± 6 mmHg and 331 ± 16 beats min^{-1}, respectively.

[Lys^7]dermorphin (0.12 to 4.73 μmol kg^{-1}, s.c.) produced a decrease in arterial systolic blood pressure of awake rats that started 5 min after peptide injection, peaked within 15 min and lasted 30 min (Figure 8). The maximum decrease (-39 ± 5 mmHg, $n=4$, $P<0.001$) in blood pressure was obtained with 1.18 μmol kg^{-1} dose, the 4.73 μmol kg^{-1} dose being less potent (-22.7 ± 3 mmHg, $n=4$, $P<0.01$ *vs* 1.18 μmol kg^{-1} dose). The dose of 14.2 μmol kg^{-1} of the peptide produced a prompt and short lasting decrease in blood pressure within 15 min of injection followed by a pressure increase that reached a maximum of $+24 \pm 7$ mmHg ($P<0.01$, $n=4$) after 60–90 min and lasted two hours. Up to the dose of 4.7 μmol kg^{-1}, the changes in blood pressure had no accompanying changes in heart rate. However, doses of 8.3 and 14.2 μmol kg^{-1} produced tachycardia to a maximum of 418 ± 18 beats min^{-1} ($P<0.05$). Naloxone (0.1 mg kg^{-1}, s.c., $n=4$) abolished all pressure changes. Naloxone methyl bromide (3 mg kg^{-1}, s.c., $n=5$) significantly decreased, though did not abolish, [Lys^7]dermorphin-induced hypotension. Naloxonazine (10 mg kg^{-1}, i.v., 24 h before, $n=4$) did not modify the depressor response.

Morphine given at 6.7 μmol kg^{-1}, s.c. slightly (-13 ± 4 mmHg, $n=8$, $P<0.05$) decreased arterial blood pressure, but higher doses of the alkaloid could not be tested because they produced marked tail rigidity that impaired blood pressure measurement by blunting the tail-artery pulse.

Discussion

Comparison of [Lys^7]dermorphin with morphine

Previous studies in rats have shown that the opioid peptide [Lys^7]dermorphin produces a potent antinociceptive response

(ED_{50} = 0.36 μmol kg^{-1}, s.c.) that reaches a maximum 90 min after s.c. injection and lasts for more than three hours (Negri *et al*., 1995). When calculated on a molar basis, the analgesic potency of [Lys^7]dermorphin is 23 times higher than that of morphine. In the present experiments s.c. doses of [Lys^7]dermorphin showed a biphasic pattern of activity on rat pulmonary ventilation. Up to the 4.73 μmol kg^{-1} (i.e., about 15 times the ED_{50} for antinociception) the peptide did not depress pulmonary ventilation in rats breathing air and, indeed, initially increased minute volume, mainly by raising respiratory frequency. Respiratory stimulation occurred earlier than antinociception, reaching a maximum 15–30 min after injection and lasting for 60 min, i.e. far less than the antinociceptive effect. Apart from this initial stimulation of ventilation, doses up to 4.73 μmol kg^{-1} produced no further respiratory changes. However, from the second hour onwards after the injection, when antinociception peaked, doses of 8.3 and 14.2 μmol kg^{-1} of [Lys^7]dermorphin decreased respiratory frequency. The fall in frequency lasted 40–50 min and was immediately balanced by a compensatory increase in tidal volume so that minute volume did not change. At these doses rats were cataleptic but yet responsive to acoustic stimulation: once stimulated they resumed their normal respiratory frequency. Similar results were also obtained with i.c.v. injection of [Lys^7]dermorphin. By this route of administration the peptide dose-dependently stimulated ventilation, but a dose (360 nmol) three times higher than that producing maximum stimulation, significantly decreased minute volume. This biphasic pattern of activity observed in s.c. injected air-breathing rats makes it difficult to calculate a reliable ratio between antinociceptive and respiratory depressant ED_{50} dose. Confirming numerous pervious findings (Kokka *et al*., 1965; Van den Hoogen & Colpaert, 1986) in awake rats, morphine exerted its peak effect on pain threshold and respiration 60 min after s.c. injection. Doses lower than 6.7 μmol kg^{-1} had no significant effects on ventilation of air-breathing rats, but the dose of 13.3 μmol kg^{-1} decreased ventilation to approximately 48% of the control level. The latter dose of morphine is only two times the alkaloid ED_{50} for antinociception (6.7 μmol kg^{-1}) and thus morphine is more likely to cause severe respiratory depression than [Lys^7]dermorphin. These results were also confirmed in rats breathing hypercapnic inspirates. In rats breathing a normoxic gas mixture containing 7% CO_2, the ratio of antinociceptive to respiratory-depressant ED_{50} dose for [Lys^7]dermorphin was 17 times lower than that for morphine. Moreover, when the CO_2 concentration in inspirates was reduced to 4%, the ratio of antinociceptive to respiratory-depressant activity of the peptide further decreased to reach a value 30 times lower than that of morphine. Thus, when analgesic doses are used, [Lys^7]dermorphin produces far less retention of CO_2 than morphine. However, when oxygen concentration was raised near to 100% in the hypercapnic inspirates, [Lys^7]dermorphin induced a more intense depression of ventilation and the ratio of antinociceptive to respiratory depressant ED_{50} dose was only 3 times less than that calculated for morphine. In contrast, all doses of the peptide tested increased the ventilator response to hypoxia (10% O_2), although this increase was larger at 1.2 μmol kg^{-1} than at 14.2 μmol kg^{-1}. In contrast, morphine at dose levels higher than 6.7 μmol kg^{-1} dose-dependently depressed the rat ventilatory response to hypoxia. In conclusion, in rats breathing air or hypoxic inspirates, respiratory depression is far less likely to occur after [Lys^7]dermorphin than after morphine.

Subcutaneous [Lys^7]dermorphin decreased arterial blood pressure in awake rats. Because we were unable to measure tail artery blood pressure in awake rats receiving doses of morphine higher than 6.7 μmol kg^{-1}, a comparison with [Lys^7]dermorphin within an equivalent dose range could not be made. However, morphine at dose of 6.7 μmol kg^{-1} produced a decrease in blood pressure equivalent to 0.12 μmol kg^{-1} [Lys^7]dermorphin.

Generation of active metabolites

[Lys^7]dermorphin is converted by rat brain enzymes to the N-terminal penta- and tetra-peptide, the last representing the minimal effective structure required to elicit the classical opioid resposes (Negri *et al*., 1992). These fragments preferentially bind to μ_2-opioid receptors and display potent opioid activity in rats (Melchiorri & Negri, 1996). In the present study, when injected i.c.v., these metabolic products promptly depressed pulmonay ventilation with a potency higher than or at least equal to that of the parent peptide. Thus the late-onset respiratory depression observed with [Lys^7]dermorphin can be ascribed, at least in part, to the generation of N-terminal fragments which preserve potent activity on μ_2-opioid receptors. In other words, *in vivo* [Lys^7]dermorphin behaves primarily as a μ_1-opioid agonist which stimulates ventilation and secondarily as a pro-drug generating μ_2-opioid agonists that are respiratory depressants.

Receptors involved in [Lys^7]dermorphin opioid activity

Previous experiments in rats have shown that [Lys^7]dermorphin produces antinociception through a selective activation of the μ_1-opioid receptor subtype (Negri *et al*., 1995). In the present experiments, the stimulation of ventilation produced by [Lys^7]dermorphin in rats breathing air was antagonized by naloxonazine as well as by naloxone pretreatment. In contrast, [Lys^7]dermorphin-induced depression of the hypercapnic ventilator response was not affected by naloxonazine and naloxone methyl bromide, although it was promptly abolished by naloxone. These results confirm previous findings in rats breathing air (Ling *et al*., 1985; Paakkari *et al*., 1990) that showed opioid-induced stimulation of ventilation to be a naloxonazine-sensitive effect (μ_1), opioid-induced depression of ventilation to be naloxonazine-resistant (μ_2) and both effects to be centrally located. In addition, the present experiments with opioid antagonists show that [Lys^7]dermorphin enhanced the ventilatory response to peripherally-sensed hypoxia through a central naloxonazine-sensitive mechanism. Thus the activation of central μ_1-receptors was an early effect that, as discussed above, could be ascribed to the intact peptide, while the activation of central μ_2-receptors was of late onset and could be due to the generation of active N-terminal fragments of the peptide.

The [Lys^7]dermorphin-induced arterial hypotension was not antagonized by naloxonazine but significantly reduced by naloxone methyl bromide pretreatment, confirming previous findings showing that opioid hypotension partially originates in the periphery (Randich *et al*., 1993).

Mechanism of [Lys^7]dermorphin-induced stimulation of ventilation

Only ventilator responses, as opposed to ventilatory sensitivity, can be assessed from inspirates. A true ventilatory sensitivity can only be determined if arterial or end-tidal gas tensions are known. In studies in which arterial and end-tidal gas tensions were measured, both in the rat (Cragg & Drysdale, 1982) and man (Lloyd *et al*., 1958), hypoxia potentiated while hyperoxia reduced the ventilatory sensitivity to hypercapnia.

Negative or positive ventilatory interactions between hyperoxia, hypoxia and hypercapnia occurred at distinct P_{O_2} to P_{CO_2} ratios in the arterial blood. Since we did not attempt to measure blood or end-tidal P_{O_2} and P_{CO_2} after opioids, we cannot exclude the possibility that stimulation of ventilation brought out by [Lys^7]dermorphin in rats breathing air or exposed to hypoxia resulted from ventilatory interactions between blood gas tensions. In any case, such interactions do not entirely explain the peptide-enhanced ventilatory responses, since naloxonazine and ritanserin antagonized the stimulation of ventilation. From the results obtained with the antagonists, it may be argued that the same mechanism underlying the stimulation of ventilation in air breathing rats operates to enhance the response to hypoxic inspirates and that [Lys^7]dermorphin apparently produces respiratory stimulation through the activation of a 5-hydroxytryptaminergic pathway.

An analysis of 5-HT receptor types involved in opioid respiratory stimulation is certainly beyond the purpose of the present work. However, in the context of the present results it is surprising that s.c. injection of ritanserin (2 mg kg^{-1}), a 5-HT$_{1D}$/5-HT$_2$/5-HT$_7$ receptor antagonist, blocked the opioid respiratory stimulation, while injections of WAY-100635 (0.3 mg kg^{-1}, s.c.), a 5-HT$_{1A}$ receptor antagonist, and GR127935 (1 mg kg^{-1}, i.v.), a 5-HT$_{1B/1D}$ receptor antagonist did not modify the opioid respiratory responses. This contrasts with the effects of activation of 5-HT$_1$ receptors located in the dorsal vagal nucleus of the rat which produced respiratory stimulation (Sporton *et al*., 1991) or of activation of 5-HT$_2$ receptors located in the IVth ventricle (Shepheard *et al*., 1991) and the ventral surface of the medulla (King & Holtman, 1990), which result in a decreased respiratory rate. Although we have not evaluated the 5-HT receptor blocking activity, the doses of 5-HT antagonists used in this study are in a similar range to those required to block central 5-HT receptors in behavioural studies (Zarrindast *et al*., 1995; Skingle *et al*., 1996; Cao & Rodgers, 1997; Clarke *et al*., 1997; Collison & Dawson, 1997; Jackson *et al*., 1997; Kleven *et al*., 1997; Meneses & Hong, 1997; Mora *et al*., 1997). A possible explanation for these apparently conflicting results is that 5-HT receptors involved in opioid respiratory stimulation are not located in the brainstem but within the forebrain. In this respect, the data from our experiments with ritanserin and metergoline are consistent with observations of Anderson *et al*. (1995) in that the activation of 5-HT$_2$ receptors in the forebrain of anaesthetized cats is excitatory to phrenic motoneurones and increases the rate of respiratory drive. Moreover the combined ritanserin and metergoline data could also indicate that 5-HT$_7$ receptors (Jasper *et al*., 1997) might be involved in respiratory stimulation, although evidence for a role for 5-HT$_7$ receptors in respiratory function is still lacking.

In addition, interactions of the BP depressor responses with respiratory effects should be considered when the mechanism of [Lys^7]dermorphin-induced respiratory stimulation is being evaluated. In the present experiments the time-course of arterial hypotension overlapped that of respiratory stimulation. However [Lys^7]dermorphin-induced hypotension was resistant to naloxonazine antagonism while respiratory stimulation was not. In contrast, the BP depressor response was reduced by pretreatment with the peripherally acting opioid antagonist naloxone methyl bromide, while ventilatory stimulation was unaffected. These results suggest that [Lys^7]dermorphin-induced stimulation of ventilation is not secondary to a blood pressure decrease.

It is concluded from the present results that [Lys^7]dermorphin affects pulmonary ventilation by two distinct mechanisms. In addition to the depression of respiration due to a decrease of carbon dioxide sensitivity of the bulbopontine respiratory centre, the opioid stimulates respiration, probably by activating a forebrain descending 5-hydroxytryptaminergic pathway which is excitatory to brainstem respiratory neurones and/or to phrenic motoneurones. The activation of this 5-hydroxytryptaminergic pathway could also explain the facilitatory influence exerted by [Lys^7]dermorphin on the respiratory response to hypoxia.

Abbreviations

f, respiratory frequency; V_t, respiratory tidal volume; V_e, respiratory minute volume; DAMGO, [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin.

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