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INVOLVEMENT OF VAGAL OPIOID RECEPTORS IN RESPIRATORY EFFECTS OF MORPHINE IN ANAESTHETIZED RATS

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Respiratory effects of morphine injection to the femoral vein were investigated in urethane and chloralose anaesthetized and spontaneously breathing rats, prior to and after midcervical vagotomy. Bolus injection of morphine HCl at a dose of 2 mg/kg of body weight induced depression of ventilation in all rats, due to the significant decrease in tidal volume and to the decline in respiratory rate both pre- and post-vagotomy. Expiratory apnoea of mean duration of 10.0 ± 3.4 s was present in the vagally intact rats only. Bilateral midcervical section of the vagus nerve precluded the occurrence of apnoea. Prolongation of the expiratory time ($T_{E \text{ morphine}} / T_{E \text{ control}}$), which amounted to 10.7 ± 2.2 -fold in the intact state, was apparently reduced to 1.5 ± 0.3 -fold after division of the vagi. Morphine significantly decreased mean arterial pressure (MAP) at 30 s after the challenge, the effect persisted for not less than 1 minute and was absent in vagotomized rats. The respiratory changes evoked by morphine reverted to the control level after intravenous injection of naloxone at a dose of 1 mg/kg. Results of this study indicate that opioid receptors on vagal afferents are responsible for the occurrence of apnoea and hypotension evoked by morphine.

Key words: control of breathing, rat, morphine, vagus nerve, opioid receptors

INTRODUCTION

Morphine, an opioid alkaloid, applied in man in the therapeutic doses depresses ventilation (1-3). It is well established that this prototype agonist of μ , activating also δ receptors, given by each route of injection (subcutaneous, intraperitoneal, intravenous, intraventricular) induces a reduction in both tidal volume and respiratory frequency in unanaesthetized rats and dogs (4-8). The

effect is generally thought to be the result of inhibition of the pontine and medullary respiratory centres via opioid receptors, distributed in the respiratory areas of pneumotaxic centre: nucleus parabrachialis medialis, Kölliker-Fuse nucleus, and in the dorsal and ventral respiratory groups (9-12).

The data referring to the presence of opioid receptors on vagal afferents are scarce.

In the rat, their expression was found on the vagus nerve, in the cell bodies of the nodose, jugular and petrosal ganglia (12-14).

As quoted above, most of the experimental schemes dealt with conscious animals and human subjects, in which the ventilatory depression and cardiovascular changes induced by morphine could not be fully measured. We have found only two reports describing the occurrence of apnoea and cardiorespiratory disturbances induced by an intravenous morphine and enkephaline challenges in decerebrated, unanaesthetized rats (15,16). The authors suggest that the pattern of the respiratory response to these opiate agonists resembles that of the pulmonary chemoreflex, engaging afferent vagal C-fibres' system.

Our objective in the present study was to re-examine the pattern of breathing evoked by morphine in the anaesthetized rats and to determine the contribution of lung vagi to the vascular and respiratory restraints. We hypothesized that respiratory depression evoked by morphine does not entirely rest on lung vagal afferentation.

MATERIAL AND METHODS

Ethical approval of the experimental procedures used in this study was obtained from the local committee. All animal procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Twelve adult male Wistar rats (weight 200 - 350 g) were anaesthetized with intraperitoneal (i. p.) injection of 600 mg/kg of urethane (Sigma, Poznań, Poland) and 120 mg/kg of alpha chloralose (Fluka AG, Poznań, Poland). They were placed supine on a heated operating table, breathing spontaneously room air. A femoral vein and femoral artery were catheterized for further injections of drugs, supplemental doses of anaesthetic and to monitor blood pressure, respectively. The trachea was divided below the larynx and the cannula inserted into its caudal end was connected to a pneumotachograph.

The midcervical segments of the vagi were separated, insulated and prepared for section later in the experiment. Tidal volume (V_T) signals were recorded from the pneumotachograph (Electrospirometer C 56, Mercury). End-tidal CO_2 was continuously monitored with an Engstrom Eliza Plus capnograph (Gambro). Arterial blood pressure was measured with a pressure transducer (CK 01 Mera-Tronik, Poland) and blood pressure monitor (MCK 4011 Mera Tronic, Poland). Electromyograms of the costal diaphragm were recorded with bipolar intramuscular needle electrodes of 0.6 mm diameter. The activity was amplified (1000-5000x) with a NL 104 amplifier (Digitimer), band-pass filtered (50Hz-50kHz) and measured with a model AS 101 (Asbit) leaky integrator (time constant = 100 ms). The recordings were registered on an Omnilight 8 M 36 apparatus (Honeywell).

Morphine HCl (Morphinum Hydrochloricum, Polfa, Kutno, Poland), dissolved in 0.9% saline was injected intravenously at a dose of 2 mg/kg: 1) in neurally intact rats and 2) in the same rats underwent midcervical vagotomy. Naloxone HCl (Naloxonum Hydrochloricum, Polfa, Warsaw, Poland) was dissolved in 0.9% saline and was given at a dose of 1mg/kg to animals, whose breathing did not restore

to the baseline one minute after morphine-induced inhibition. Recovery from naloxone lasted 60-90 min, and after this time next morphine challenge could have been done. Control injection of 0.9 % NaCl alone produced no effect on the recorded respiratory variables. Each individual value of V_T , ventilation (V_E) and respiratory rate (f) was taken as an average of five consecutive breathes. The ventilatory parameters were assessed prior to the morphine injection, at the early post-apnoeic phase (the first five post-apnoeic breathes) and at 30 and 60 s after the challenge. The expiratory time (T_E) was determined from the record of integrated diaphragm activity (from the end of descending phase of diaphragmatic integral to the onset of the following rising phase). T_E prolongation was measured as the ratio of maximal T_E after morphine challenge ($T_{E\text{ morphine}}$) to the respective control T_E value ($T_{E\text{ control}}$). The duration of apnoeic period in the diaphragmatic activity was measured as the time of apnoea.

Each individual value of V_T , V_E , f and T_E were analyzed by two-way ANOVA with post-morphine time (0, early post-apnoeic phase, 30 s and 60 s) as repeated measures' factor and the innervation status (intact and vagi cut) as a between condition factor. Differences between individual time points and experimental situations were evaluated by contrast analysis. In all cases, a $p < 0.05$ was considered significant. The results shown are means \pm S.E.M.

RESULTS

Selection of the dose of morphine used in the present experiments was based on the dose-response relationships shown in *Fig. 1*, which indicate 2 mg/kg to be most effective in inducing the ventilatory depression.

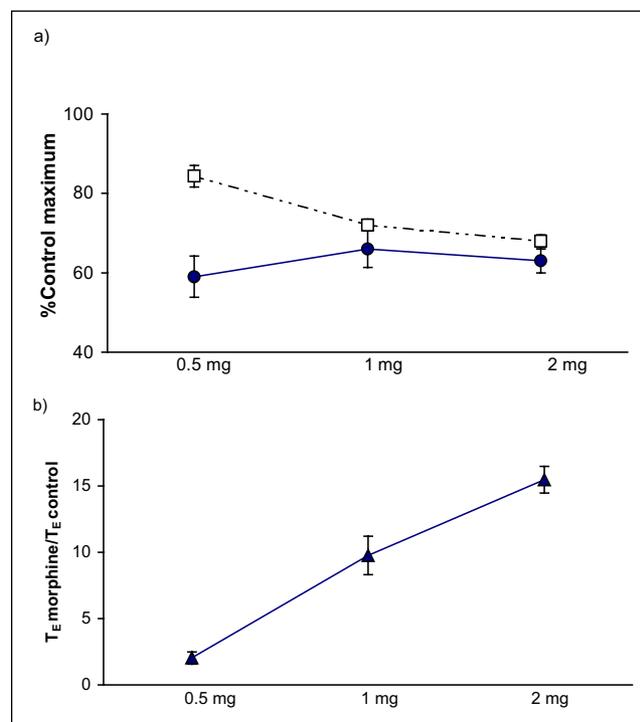


Fig. 1. Dose-response curves. (a) The percentage of maximal possible effect of morphine referenced to control values: on tidal volume (●) and frequency of breathing (□). (b) The relationship between the dose of morphine and mean prolongation of the expiratory time (T_E). Data are mean \pm S.E.M., $n=6$ (one-way ANOVA).

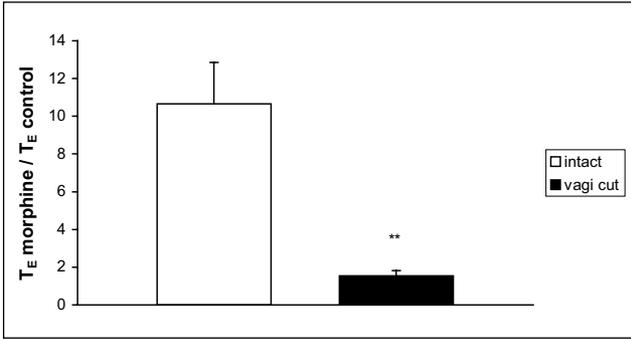


Fig. 2. Effect of midcervical vagal section on the mean prolongation of T_E induced by an intravenous morphine injection. (mean \pm S.E.M., $n = 12$), ** $p < 0.01$, vs pre-vagotomy values (one-way ANOVA).

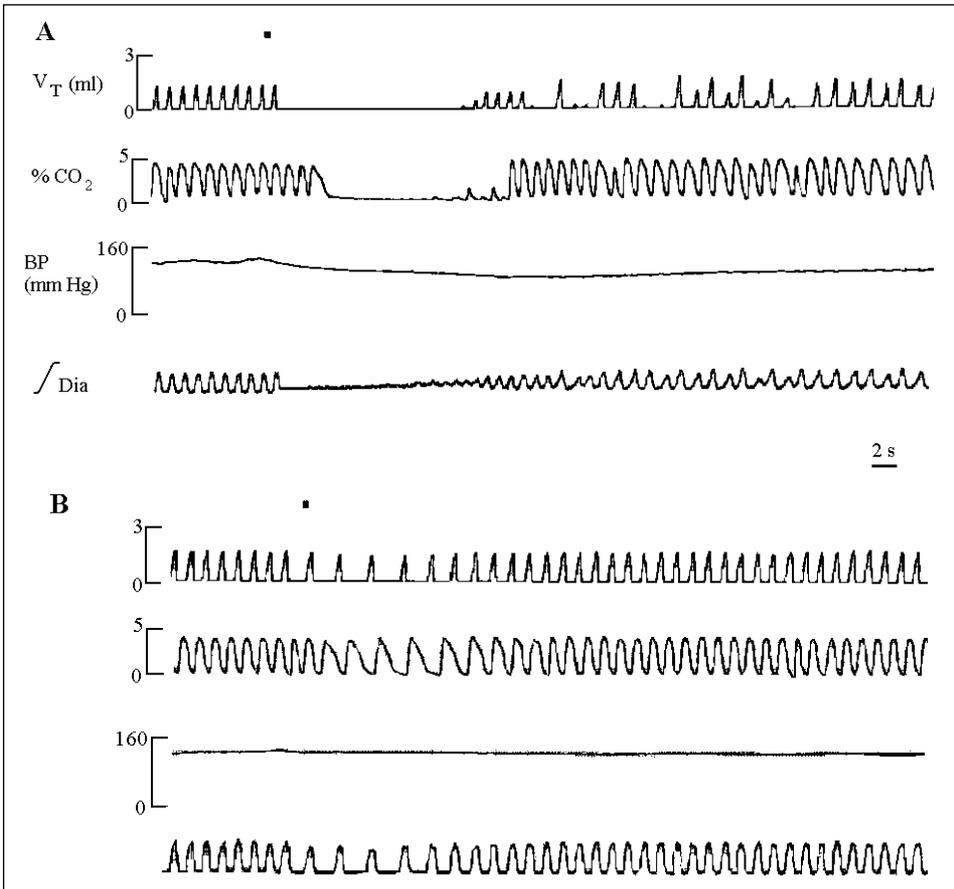


Fig. 3. Respiratory response to i.v. morphine challenge in vagally intact rat (A). Injection marked by a dot above the upper record. Note an apnoea, followed by slowing down of breathing with decreased tidal volume and a fall in blood pressure. Midcervical vagotomy (B) abolished the respiratory apnoea and the decrease in blood pressure.

V_T , tidal volume; $\%CO_2$, end-tidal CO_2 ; BP, arterial blood pressure; \int Dia, integrated electromyogram of the diaphragm.

In initially neurally intact and subsequently vagotomized rats intravenous injection of morphine produced uniform respiratory effects, comprising prolonged depression of respiration due to the decreased tidal volume and to the decline in the frequency of breathing, and a fall in blood pressure. All rats, prior to vagotomy, responded with an expiratory apnoea of mean duration of 10.0 ± 3.4 s. In the apnoeic pause, the expiratory time was significantly elongated; the mean prolongation of T_E being 10.65 ± 2.21 -fold and division of the vagi entirely precluded this effect to 1.54 ± 0.28 -fold ($p < 0.01$) (Fig.2). Fig.3 shows the typical respiratory response to an intravenous morphine administration in vagally intact (A) and subsequently vagotomized rat (B).

Morphine challenge showed significant effect on tidal volume ($p < 0.05$, two-way ANOVA). In the neurally intact rats, in the initial phase of renewed post-apnoeic breathing, V_T fell significantly ($p < 0.05$), compared to pre-drug controls. During 30 and 60 s after the challenge V_T returned to the control value. Following section of midcervical vagi, tidal volume decreased immediately after injection of opiate ($p < 0.001$) and the fall was prolonged and persisted until 60 s ($p < 0.01$). Vagotomy in itself increased significantly mean control value of V_T ($p < 0.0001$), (Table 1).

Frequency of breathing was affected by morphine treatment and diminished significantly in both: neurally intact and vagotomized animals ($p < 0.0000001$, two-way ANOVA). The slowing down of the respiratory rate was present immediately after morphine challenge and lasted until 60 s both before and after vagotomy. In the latter state, minor decreases in the respiratory rate produced by morphine were result of the lower initial control value (Table 1).

Minute ventilation was significantly affected by the drug challenge ($p < 0.000001$, two-way ANOVA). The effect of decreased V_E was present in the initial post-morphine phase and remained significantly depressed until 60 s after injection in both neural states (Fig. 4).

Post-morphine respiratory disturbance was coupled with the fall in mean arterial blood pressure in the intact state, observed at 30 and 60 s after injection. The maximum, highly significant drop from the control value of 119 ± 7.7 to 98 ± 9.8 mmHg was present at 30 s ($p < 0.01$). After section of the vagi, morphine induced immediate small insignificant rise in MAP from 109 ± 7.0 to 112 ± 9.7 mmHg.

Table 1. Changes in tidal volume (V_T) and respiratory rate (f) after intravenous morphine challenge

Innervation status	V_T (ml)				f (min^{-1})			
	Baseline	After morphine			Baseline	After morphine		
		Early phase	30 s	60 s		Early phase	30 s	60 s
intact	1.80 ± 0.1	$1.54 \pm 0.2^*$	1.89 ± 0.2	1.93 ± 0.2	59 ± 2.8	$48 \pm 3.7^{**}$	$45 \pm 3.1^{***}$	$44 \pm 2.6^{***}$
vagi cut	$2.41 \pm 0.2\#$	$2.13 \pm 0.2^{***\#}$	$2.15 \pm 0.2^*$	$2.10 \pm 0.2^{**}$	$50 \pm 3.3\#$	$43 \pm 4.5^*$	$47 \pm 4.1^*$	$47 \pm 4.1^*$

All values are means \pm S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the respective pre-morphine value. # $p < 0.001$ vs. the corresponding pre-vagi cut value. Two-way ANOVA followed by planned contrasts' analysis, $n=12$

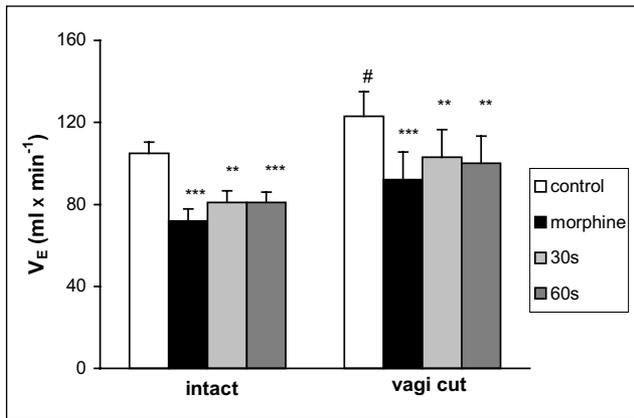


Fig. 4. Effect of i.v. morphine injection on minute ventilation (V_E) in the intact and vagotomized rats.

** $p < 0.01$, *** $p < 0.001$ vs. the respective pre-morphine value; # $p < 0.05$ vs. the corresponding pre-vagotomy value, $n = 12$ (two-way ANOVA).

Intravenous injection of naloxone, an opiate receptors' antagonist, applied when necessary, readily reverted (within seconds) the extended depression of V_T and f induced by morphine to the control values.

DISCUSSION

The present study describes changes in pulmonary parameters and blood pressure induced by stimulation of opioid receptors. In the intact rats an intravenous injection of morphine evoked prolonged respiratory depression due to the immediate arrest of breathing, decline in tidal volume and respiratory rate.

Our results are in general agreement with previously published data. Morphine given subcutaneously in awake, intact rats produced decrease in the frequency of breathing and tidal volume (3,17). These effects were much slower in onset and tended to last longer, compared to systemic challenge. The difference was probably due to the route of administration and to the dose used (10-160 mg/kg), which was many times higher than the one used in our study.

Willette and Sapru (15,16) using the same dose of morphine as ours or enkephalines (250 $\mu\text{g}/\text{kg}$) injected into the right atrium in unanaesthetized, and decerebrated intact rats, reported an apnoea followed by prolonged, rapid shallow breathing of increased minute ventilation. The pattern of the respiratory response fitted to and was shown to depend on activation of pulmonary C fibres afferent system. To the contrary, the evidence presented here shows that apnoea induced by an intravenous injection of morphine was succeeded by the short-lived fall in V_T , concomitant with extended decrease in the breathing rate, resulting in a progressive hypoventilation. The responses were well established and consistent. This divergence may be related to the different derivative of morphine used in the quoted study (morphine sulphate). Morphine hydrochloride used in our study was shown to be very effective in evoking the depression of ventilation in rats (18).

Next, the difference in the pattern of post-apnoeic breathing may be caused by decerebration used by Willette and Sapru (15,16). This intervention does not require anaesthesia and excludes its depressive effects, but reduces the blood supply to the brainstem.

In the current study, division of the vagi in the neck precluded the arrest of breathing but showed no effect on alterations in the respiratory rate and V_T . This denotes that only apnoea constitutes the reflex arising from activation of vagal afferent system in the lungs, which confirms in this aspect previous evidence, when vagi were sectioned below the cardiac branches (15,16).

Further, as revised in Introduction rat vagal afferents possess opioid receptors (13). Basing on previously published results, morphine within the reach of pulmonary circulation increased lung and laryngeal resistances during the arrest of breathing, which was abolished by midcervical vagotomy and section of the recurrent laryngeal nerves (respectively) and pre-treatment with naloxone (19,20).

We have excluded the possibility that the lack of apnoea on the second morphine injection after vagotomy was a sign of tolerance. It is reinforced by the identical response to morphine in the intact rats, which received two injections of the drug.

In our experiments naloxone was effective in reverting the depression of breathing evoked by morphine, thereby implying contribution of the opioid receptors. It is of note that we have applied naloxone hydrochloride, which crosses blood-brain barrier (21). Having this knowledge we may suspect that the same pattern of the respiratory response in both neural states (apnoea excluded) results from stimulation of central or extrapulmonary structures supplied with opioid receptors. These latter are associated with the sensory neurones of the nodose, jugular and petrosal ganglia (12). However we cannot confirm our speculation because the vagal input to the brainstem via the nodose and jugular ganglia was not disrupted in our experiments, similarly to that from the carotid bodies.

Morphine penetration into the brain has been reported rather limited (21) but we cannot exclude potentially suitable central effect of the drug. Depression of V_E , V_T and f evoked by morphine in our vagotomized rats is in line with the effects obtained after application of the drug to the ventral areas of the medulla oblongata in anaesthetized cats (22) and following fourth ventricular microinjection in rats (23). Also μ opioid receptors in the brainstem contribute to opioid-evoked depression of breathing in neonatal and adult rats (24).

The drop in mean arterial pressure evoked by peripheral morphine administration observed in our study, falls in line with the previously obtained results on its cardiovascular effects (25,26). Bilateral section of cervical vagi precluded hypotension (16) and converted the initial depressor response to morphine into the pressor effect (26,27). On the other hand, intracisternal morphine injection in anaesthetized rats evoked the rise in blood pressure (28). We cannot therefore exclude that the pressor response, registered in our experiments after vagal section, reflects the central action of morphine.

The results of the present study confirm that morphine-induced apnoea and the fall in blood pressure are mediated by an opioid receptor mechanism on the lung vagal pathways. Our findings show that morphine-induced respiratory depression affects the respiratory pattern beyond the intrathoracic vagi.

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