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Short communication

Supranodose vagotomy eliminates respiratory depression evoked by dermorphin in anaesthetized rats

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Abstract

The respiratory effects of stimulation of μ-opioid receptors were studied in spontaneously breathing anaesthetized rats that were either neurally intact or subjected to bilateral supranodosal vagotomy. An intravenous dermorphin bolus of 0.5 mg/kg evoked the apnea followed by breathing of reduced rate and compensatory augmentation of tidal volume, which resulted in an invariable minute ventilation. Cardiovascular effects consisted of hypotension and temporary fall in heart rate.

In rats initially treated by supranodosal vagotomy, dermorphin did not evoke any respiratory and cardiovascular effects.

These results indicate that vagal pathway and the nodose ganglia are involved in dermorphin-induced respiratory depression.

Keywords: Dermorphin; Respiration; Nodose ganglia

1. Introduction

Respiratory depression is a serious problem in clinical use of opioid analgesics. Of these, dermorphin, a natural opioid isolated from the skin of frog, is a potent selective μ-agonist, affecting both central and peripheral receptors depending on the route of application (Melchiorri and Negri, 1996).

The opioid receptors are widely distributed in the respiratory areas of the pneumotaxic center: nucleus parabrachialis medialis, Kőlliker–Fuse nucleus, and in the dorsal and ventral respiratory groups of the medulla (McQueen, 1983; Nomura et al., 1996; Sales et al., 1985). The expression of opioid receptors was found on the vagus nerve, in the cell bodies of the nodose, jugular and petrosal ganglia of the rat (Ding et al., 1998; Li et al., 1996; Zarbin et al., 1990; Zhuo et al., 1998).

Dermorphin applied intracerebroventricularly (i.c.v.) to the conscious rats has been shown to increase minute volume and respiratory rate; higher doses decreased both ventilatory subdivisions (Paakkari et al., 1990). In the similar experimental conditions, dermorphin induced the decrease in minute ventilation due to reduction in respiratory rate and a delayed rise in tidal volume (Colman and Miller, 2001). In anaesthetized rats i.c.v. dermorphin challenge produced the apnea (Portolano et al., 1991), which was also displayed after an intravenous injection, and followed by the depression of tidal volume with no change in the frequency of breathing (Eager et al., 1994).

The effects of dermorphin on blood pressure were less extensively searched. Intravenous challenge in anaesthetized rats induced a fall in systemic blood pressure and a moderate bradycardia (Eager et al., 1994; Melchiorri and Negri, 1996; Portolano et al., 1991).

In view of these few and divergent results, the purpose of the present investigation was to examine more directly the effects of the systemic dermorphin challenge on the respiratory pattern and cardiovascular response and to assess whether vagal afferentation to the medulla is the main reflex pathway engaged.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (150–180 g body weight) were anaesthetized with an intraperitoneal injection of 750 mg/kg urethane (Sigma) and 150 mg/kg α-chloralose (Fluka AG). Supplementary urethane doses were administered intravenously

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(i.v.) as indicated by response(s) to nociceptive test stimuli. All animal use procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local ethics committee.

2.2. Surgical procedures

The animals were placed supine recumbency and breathed room air spontaneously. The trachea was exposed in the neck, sectioned below the larynx and cannulated. Catheters were inserted into the femoral vein for drug administration and into the femoral artery for blood pressure monitoring. Rectal temperature was maintained at 38 °C with a heating pad. In the second group of animals, the rostral parts of midcervical vagi were separated from the superior cervical ganglia. The nodose ganglia were bluntly dissected from the surrounding tissue; attention was paid to preserve their blood supply intact. The supranodose vagi were transected 2 mm distal from the rostral poles of the ganglia, prior to measuring the respiratory variables.

2.3. Apparatus and recordings

The tracheal cannula was connected to a pneumotachograph head, linked to Research Pneumotach System (RSS 100 HR, Hans Rudolph Inc.) and a computerized recording system (Windows software version 3.07.02, KORR Medical Technologies Inc.) for measuring and recording tracheal airflow, respiratory frequency (f), tidal volume (V_T), respiratory minute volume (V_E), inspiratory (T_I) and expiratory (T_E) times. Arterial blood pressure was measured with a BP-2 monitor (Columbus Instruments).

Electromyogram of the costal diaphragm was recorded with bipolar electrodes connected to a model NL 104 amplifier (Digitimer), and filtered and measured with a model AS 101 (Asbit) leaky integrator (time constant, 100 ms).

The recordings were registered with an Omnilight 8M 36 apparatus (Honeywell).

2.4. Drugs and treatments

Dermorphin has been synthesized in our laboratory. Analytical properties of the synthesized peptide were identical with the published data.

The respiratory effects of dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) challenge were tested in two separate groups of animals, being administered as a single dose in each rat

(i) neurally intact rats ($n=10$)
(ii) treated by bilateral vagotomy at the supranodosal level ($n=11$)

In both experimental groups dermorphin was injected into the femoral vein at a dose of 0.5 mg/kg. The dose was derived from the initial dose–response relationship (not shown). Each drug bolus was immediately flushed with 0.2 ml of physiological saline.

2.5. Measurements

The ventilatory response parameters were assessed over the first five breaths just before drug challenge, immediately after the post-challenge apnea, 15, 30, 60, 120 s and 10 min after the challenge. Mean arterial pressure was calculated and heart rate recorded in the same time intervals. T_E prolongation was measured as the ratio of maximal T_E during post-drug apnea (or expiration) to the respective control T_E value ($T_{E\ drug}/T_{E\ control}$). The duration of apneic period in diaphragm activity or V_T was used as an index of respiratory inhibition.

2.6. Statistical analysis

V_T, V_E, f and T_E data were first analysed by two-way ANOVA with repeated measures on post-dermorphin challenge time (pre-challenge, early post-apneic phase, and 15, 30, 60, 120 s and 10 min after the challenge) and on innervation status (intact, supranodose vagi cut). Differences in the ventilatory parameters between various time points and innervation states, and T_E prolongation were evaluated by Student t-test for paired data when appropriate (with Bonferroni correction for multiple comparisons). In all cases, a $P \leq 0.05$ was considered significant. The results shown are means ± S.E.M.

3. Results

In neurally intact rats, injection of physiological saline (0.2 ml) did not affect respiration (not shown), while an i.v.

Table 1
Changes in tidal volume (V_T) and respiratory rate after intravenous dermorphin challenge in neurally intact ($n=10$) and vagotomized at supranodosal level rats ($n=11$)

	Baseline	After dermorphin				
		15 s	30 s	1 min	2 min	10 min
V_T (ml)						
Intact	1.65±0.1	2.1±0.2	2.2±0.2[a]	2.2±0.2[a]	2.1±0.2	1.9±0.1
s.n. vagotomy	2.12±0.14	2.1±0.13	2.11±0.13	2.13±0.13	2.18±0.13	2.26±0.13
Respiratory rate (breaths/min)						
Intact	75.0±2.0	47.0±5.2[b]	53.0±4.2[c]	53.0±3.8[c]	57.5±5.0[b]	64.2±4.0
s.n. vagotomy	39.8±3.5	41.0±4.2	39.7±3.5	38.6±3.3	38.5±3.3	41.0±3.5

Two-way ANOVA revealed: (i) significant effects of dermorphin ($P<10^{-6}$) and dermorphin × vagotomy interaction effect ($P<10^{-6}$), but no effect of vagotomy ($P=0.41$) on V_T, and (ii) significant effect of dermorphin ($P<10^{-6}$), vagotomy ($P<0.006$) and dermorphin × vagotomy interaction effect ($P<10^{-6}$) on respiratory rate. All values are means ± S.E.M. [a] — $P<0.05$, [b] — $P<0.001$, [c] — $P<0.0001$ versus the respective pre-dermorphin value. s.n. — supranodose.

Table 2
Changes in mean arterial pressure (mmHg) and heart rate (beats/min) after intravenous dermorphin challenge

	Baseline	After dermorphin			
		Apnea	30 s	1 min	2 min
Mean arterial pressure					
Intact ($n=10$)	96.1±4.74	66.6±4.8[b]	65.1±7.2[c]	75.0±5.8[b]	80.3±5.3[a]
s.n. vagotomy ($n=10$)	69.8±6.7	75.5±7.9[a,e]	75.7±8.7	69.8 ±7.8	63.6±6.8
HR					
Intact ($n=9$)	388.0±19.2	324.5±20.0[d]	367.0±25.2	376.5±19.0	367.0±23.3
s.n. vagotomy ($n=11$)	470.0±14.4	457.6±18.7[a,e]	468.8±14.9	467.5±13.0	466.0±13.0

Two-way ANOVA showed: (i) significant effect of dermorphin ($P<0.001$) and dermorphin × vagotomy interaction effect ($P<10^{-6}$), but no effect of vagotomy ($P=0.22$) on mean arterial pressure, and (ii) significant effect of dermorphin ($P<0.001$) and vagotomy ($P<0.001$) but no dermorphin × vagotomy interaction effect ($P=0.05$) on heart rate.
All values are means±S.E.M. [a] — $P<0.05$, [b] — $P<0.01$, [c] — $P<0.001$, [d] — $P<0.0001$ versus the respective baseline value. Two-way ANOVA followed by *t*-test for paired data. s.n. — supranodose, [e] — immediately after dermorphin.

dermorphin injection produced significant respiratory effects. The effects invariably comprised an apnea followed by breathing of reduced rate and increased V_T. The responses were well established and consistent.

Dermorphin injected at a dose of 0.5 mg/kg evoked apnea of mean duration of 10.4±1.74 s ($P=0.0003$). In the apneic pause, the expiratory time was significantly elongated; the mean prolongation of T_E being 19.7±3.6 folds ($P=0.0005$).

As shown in Table 1, after recovery from apnea the respiratory rate decreased and persisted at a significant level at 2 min post-challenge, returning to the baseline value 20 min after injection of dermorphin. The concomitant increase in V_T achieved significance at 30 s after dermorphin and remained insignificantly elevated within 20 min. The insignificant decrease in V_E appeared immediately after dermorphin injection and at the next post-challenge time point reached the baseline level (not shown). Supranodose vagotomy precluded the occurrence of all respiratory responses to administration of dermorphin.

As shown in Table 2, dermorphin challenge induced significant decrease in mean arterial pressure, initiated in the apneic spells and maintained at 2 min. The decrease in heart rate was short-lived and related exclusively to the period of apnea. After supranodose vagotomy injection of dermorphin induced a significant rise in mean arterial pressure immediately after the challenge.

4. Discussion

This study showed that the principal effect of an i.v. dermorphin challenge in the neurally intact rats was a prompt apnea followed by a prolonged slowing of the respiratory rhythm, associated with augmented V_T. Previous studies, already mentioned in the Introduction, yielded heterogeneous data related to the dose and route of drug administration and consciousness of the rats. The regular apnea revealed in our study is consistent with the respiratory effects of centrally and intravenously applied dermorphin (Eager et al., 1994; Portolano et al., 1991).

The initial suppression of central inspiratory activity induced by an intravenous dermorphin in the current experiments may depend on several factors. Excitation of the central receptors seems questionable, since dermorphin, most peptides alike, has very low blood brain permeability index (Fiori et al., 1997). Then the respiratory changes observed in our study likely involve peripheral effects.

Apnea is assumed to arise from stimulation of vagal sensory receptors in the lungs. The respiratory sequelae induced by dermorphin retained the regular apnea, straying from the breathing pattern typical for C fiber activation (Lee and Pisarri, 2001). Delayed increase in tidal volume (see Table 1), might be secondary to the reduction in apnea, which normally masks part of the hyperventilation. The depressive effects of dermorphin on respiratory frequency and compensatory increase in V_T maintaining minute ventilation unchanged we found, are in general agreement with the results of Negri et al. (1998) obtained in awake rats treated by subcutaneous injection of [Lys^7]dermorphin.

It is assumed that both respiratory frequency and tidal volume are regulated by μ receptors (Haji et al., 2000). Dermorphin, highly selective μ-opioid receptors agonist, while reaching pulmonary circulation after i.v. challenge, activates these receptors expressed on vagal afferents and the respiratory response is μ-mediated. Opioid receptors are likely to be inhibitory to the activation of the sensory nerves (Barnes, 1992). This may result in the depression of breathing and pattern of response which is not characteristic for any type of vagal endings in the lungs. In the current experiments we did not apply any selective blocker intentionally, as our goal was to determine the extrapulmonary structures involved in the respiratory response. Based on our experience from the initial experiments and published results (Negri et al., 1998), we were aware that only single dermorphin challenge in the rat evokes an effective cardiorespiratory response. Lack of any effect on the second trial, performed after several hours might suggest, recently discussed, rapid desensitization or internalization of μ-opioid receptors (Bailey and Connor, 2005). Considering that only one dose of dermorphin can be applied in each rat, we did not hesitate to neglect the specific blockade of the peripheral receptors and separated the supranodosal connection to the medulla. Furthermore, it was shown that vagal neurotomy at the lower, midcervical level precluded post-morphine apnea in anaesthetized rats, while

the ventilatory depression, affecting both tidal and timing components of the breathing pattern, occurred beyond the intrathoracic vagi (Kaczyńska and Szereda-Przestaszewska, 2005).

This is the first report comparing the respiratory response to dermorphin administered via peripheral circulation in the neurally intact rats with those neurotomized at the supranodose level. Disconnection of the central vagal trunk above the nodose ganglia increased baseline tidal volume and lowered the frequency of breathing, which is consistent with the effects of interruption of the vagal feedback. Supranodose vagotomy, damaging the vagal input to the brainstem excluded the effects of dermorphin on ventilatory components (Table 1). This implies that the nodose ganglia are essential in the reflex response to dermorphin. As noted in the Introduction, rat's nodose ganglia are richly supplied with μ receptors (Zhuo et al., 1998). The presence of μ receptors on the vagus nerve is indicative of a possible axonal transport of these receptors in both the central and peripheral ramifications of the vagus nerve (Ding et al., 1998; Li et al., 1996; Zarbin et al., 1990). Separation of the nodose ganglia interrupts their central projection and disturbs dermorphin regulation of the peripheral reflex.

The fall in blood pressure in rats with preserved supranodose connection falls in line with the previous observations on hypotensive effects of dermorphin (Eager et al., 1994; Melchiorri and Negri, 1996; Portolano et al., 1991). They were described to be related to an activation of peripheral opioid receptors on vagal afferents (Negri et al., 1998; Randich et al., 1993). Short-lived hypertensive response following damage of the vagal pathway to the brainstem (see Table 2) is likely to be the consequence of aortic baroreceptors denervation.

In summary this study has shown that i.v. dermorphin challenge consistently produces apnea, depresses ventilation, primarily due to the large decrease in respiratory rate. The depression is executed most probably via activation of μ-opioid receptors on the vagal pathway to the brainstem and the nodose ganglia are the crucial point for the reflex respiratory effect of systemically given dermorphin in anaesthetized rats.

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