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Respiratory and cardiovascular effects of biphalin in anaesthetized rats

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ABSTRACT

Biphalin (0.3 mg/kg) administered intravenously (i.v.) to urethane-chloralose anaesthetized rats consistently evoked apnoea, followed by breathing at subnormal respiratory rate with increased tidal volume. Mean arterial pressure and heart rate were lowered. Naloxone completely antagonized the respiratory and cardiovascular responses to biphalin. Midcervical vagotomy prevented all respiratory effects of biphalin, and nearly abolished the fall in blood pressure and attenuated bradycardia. These results indicate that μ opioid receptors distributed in areas supplied by vagal afferents (e.g. the lung) are involved in respiratory and hypotensive effects of biphalin, whereas bradycardia may be explained by activation of brainstem regions mediating cardiovascular control.

1. Introduction

Biphalin, first synthesized by Lipkowski et al. (1982), is a dimer comprised of two opioid active tetrapeptide enkephalin analogues connected through a hydrazide bridge. Biphalin expresses almost equal affinity for μ and δ receptors (Misicka et al., 1997). Although, its affinity for the μ receptor is similar to that of morphine, its antinociceptive activity in animal models is much greater than morphine after intracerebroventicular (Horan et al., 1993) or intrathecal administration (Kosson et al., 2005, 2008). Biphalin partially crosses both the blood–brain and blood–cerebrospinal fluid barrier (Abbruscato et al., 1997).

Each of the two tetrapeptide moieties of biphalin is an analogue of the enkephalins, endogenous opioid peptides contained in the neuronal cell bodies in subnuclei of the nucleus tractus solitarii in rats (Armstrong et al., 1981) and present as well in the ponto-medullary respiratory neurons in cats (Denavit-Saubié et al., 1978). In contrast to δ receptors, μ receptors are densely distributed within the respiratory areas of the brain (Mansour et al., 1995) and outside the brain, in the vagus nerve and its ganglia (Ding et al., 1998) as well as in the adrenal medulla.

It is generally accepted that opioid agonists applied to the cerebral ventricles, nucleus tractus solitarii or ventrolateral medulla induce respiratory depression in rats. Few published findings on the effects of separate challenges with either μ or δ receptor agonists demonstrate decreases in the frequency of breathing (Pazos and Florez, 1983) tidal volume (Hassen et al., 1982), or a combination of mechanisms (Chen et al., 1996). Indeed, the synthetic enkephalin — FK-33–824, appeared to evoke hypoventilation affecting both components of ventilation (Rabkin, 1991a,b).

Several attempts have been made to assess whether μ enkephalinergic receptors are involved in the peripheral control of breathing. Intraperitoneal administration of μ or δ receptor ligands reduces either tidal volume or respiratory rate in rats, respectively (Morin-Surun et al., 1984). Further, systemic injections of DAMGO, an enkephalin analogue, decreases minute ventilation due to reduction in respiratory rate including the occurrence of apnoeic intervals in anaesthetized rats (Czapla et al., 2000).

The sole report to date on the effects of an intraperitoneal biphalin challenge on respiration in rats shows an inhibition of both components of the respiratory pattern: tidal volume and frequency of breathing (Kamei and Kasuya, 1988).

Cardiovascular effects of μ receptor activation by enkephalinergic ligands have been less extensively searched. In rats, FK 33–824 applied to the fourth cerebral ventricle evoked hypotension and bradycardia (Rabkin, 1991a,b). Systemic challenge with DAMGO produced the same pattern of cardiovascular response (Czapla et al., 2000).

To date, therefore no clear picture of biphalin effects upon the cardio-respiratory responses has emerged. The present experiments were performed to determine how activation of peripheral opioid receptors with biphalin influences the respiratory pattern, and to what extent these responses depend on lung vagal afferentation.

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 doses were

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Table 1
Changes in tidal volume (V_T) and respiratory rate caused by i.v. biphalin challenge before and after midcervical vagotomy in rats ($n=8$)

	Baseline	After biphalin				
		15 s	45 s	1 min	2 min	10 min
V_T (ml)						
Intact	1.6±0.1	2.7±0.3[a]	2.7±0.2[b]	2.6±0.2[b]	2.5±0.2[b]	2.0±0.2
Midcervical vagotomy	2.9±0.2[g]	2.7±0.2[e]	2.8±0.2	2.9±0.2	2.9±0.2	2.9±0.2[f]
Respiratory rate (breaths/min)						
Intact	73.8±3.7	38.1±3.4[c]	41.0±3.3[c]	41.7±3.7[c]	44.4±4.3[c]	59.6±4.8
Midcervical vagotomy	39.0±2.2[g]	39.0±2.4	38.7±2.4	38.3±2.3	38.6±2.3	39.2±2.3[f]

Two-way ANOVA revealed: (i) significant effects of biphalin ($F_{5,70}=15.04$, $P<0.00001$) and biphalin×vagotomy interaction effect ($F_{5,70}=18.59$, $P<0.00001$), but no effect of vagotomy ($F_{1,14}=3.51$, $P=0.082$) on V_T, and (ii) significant effect of biphalin ($F_{5,70}=39.47$, $P<0.00001$), vagotomy ($F_{1,14}=7.37$, $P<0.05$) and biphalin×vagotomy interaction effect ($F_{5,70}=37.58$, $P<0.00001$) on respiratory rate.
All values are means±S.E.M. a— $P<0.05$, b— $P<0.01$, c— $P<0.001$ versus the respective pre-biphalin value, and e— $P<0.05$, f— $P<0.01$, g— $P<0.001$ versus the respective pre-vagotomy value.

administered intravenously (i.v.) as indicated by response (s) to nociceptive test stimuli. All animal 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

Fully anaesthetized rats were placed in the supine position, where they spontaneously breathed room air. The trachea was exposed in the neck, sectioned below the larynx and cannulated with 1.8–2.4 mm gauge polyethylene tubing. Catheters (0.5–0.8 mm gauge) were inserted into the femoral vein for drug administration and into the femoral artery for blood pressure monitoring. Core body temperature was maintained between 36 and 38 °C with a heating pad. The midcervical vagi were bluntly dissected and prepared for bilateral vagotomy prior to measuring the studied variables in neurally intact rats.

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).

The 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 archived using an Omnilight 8M 36 apparatus (Honeywell).

2.4. Drugs and treatments

Biphalin was synthesized in our laboratory. Analytical properties of the peptide have been already described (Lipkowski et al., 1982; Egleton et al., 1998).

The respiratory effects of biphalin (Tyr-D-Ala-Gly-Phe-NH)$_2$ challenge were tested in two separate groups of animals, following administration as a single or double dose in each rat:

(i) before and after bilateral midcervical vagotomy ($n=8$)
(ii) after the blockade with naloxone hydrochloride (Sigma), administered i.v. at a dose of 1 mg/kg, 2 min prior to biphalin injection in the intact rats ($n=8$).

In both experimental groups biphalin was injected into the femoral vein at a dose of 0.3 mg/kg. The dose was derived from pilot dose-response studies (not shown). Each drug bolus was immediately flushed with a 0.2 ml aliquot of normal saline.

2.5. Measurements

Ventilatory parameters were assessed over the first five breaths just before drug challenge, immediately after the post-challenge apnoea, 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 apnoea (or expiration) to the respective control T_E value ($T_{E\ drug}/T_{E\ control}$). The duration of the apnoeic period as indicated by diaphragm electromyographic activity 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-biphalin challenge time (pre-challenge, early post-apnoeic phase, and 15, 30, 60, 120 s and 10 min after the challenge) and on innervation status (intact or midcervical vagotomy) or naloxone pre-treatment (yes or no) as factors of repeated measures. Differences in the ventilatory parameters between various time points and innervation states, and T_E prolongation were evaluated by Student's t-test for paired data when appropriate (with Bonferroni correction for multiple comparisons). In all cases, a $P\leq0.05$ was considered significant. The results shown are means±S.E.M.

3. Results

In the neurally intact rats, i.v. biphalin challenge produced uniform respiratory effects, of immediate apnoea followed by breathing at a decreased respiratory rate and increased tidal volume. Biphalin injected at a dose of 0.3 mg/kg evoked apnoea of mean duration of 13.5±1.25 s ($P<0.0001$, $n=8$). In the apnoeic phase, the expiratory time was elongated, the mean prolongation of T_E being 24.6±3.1 folds ($P<0.001$). As shown in Table 1, biphalin evoked significant increases in V_T from the immediate post-apnoeic phase to the later time points, reaching nearly the baseline value at 30 min. Biphalin concomitantly produced a significant reduction in respiratory rate, returning to the initial value at 30 min. Minute ventilation was decreased following biphalin challenge (ANOVA, $P<0.0001$). The decrease in V_E appeared immediately after apnoea but reached statistical significance at 1 min 15 s post-challenge (not shown).

Table 2
Mean blood pressure and heart rate changes to i.v. biphalin challenge ($n=8$)

	Baseline	After biphalin				
		15 s	1 min	2 min	10 min	20 min
Mean arterial pressure (mm Hg)						
Intact	110.2±4.8	45.8±2.9[d]	77.3±8.6[b]	81.7±7.5[b]	95.0±7.6[a]	99.8±8.1
Midcervical vagotomy	87.3±8.2[e]	84.3±7.8[g]	76.8±7.5	76.0±6.9	87.1±5.5	89.0±5.7
Heart rate (beats/min)						
Intact	432.4±11.5	316.9±11.6[d]	365.5±17.5[a]	361.9±18.6[a]	412.6±18.4	451.4±9.9
Midcervical vagotomy	465.5±15.0	454.6±16.1[h,b]	448.1±16.2[f,c]	447.9±18.0[f,b]	467.8±19.9	476.9±19.0

Two-way ANOVA showed: (i) significant effect of biphalin ($F_{5,70}=24.25$, $P<0.00001$) and biphalin×vagotomy interaction effect ($F_{5,70}=16.50$, $P<0.00001$), but no effect of vagotomy ($F_{1,14}=0.03$, $P=0.86$) on mean arterial pressure, and (ii) significant effect of biphalin ($F_{5,70}=32.76$, $P<0.00001$), vagotomy ($F_{1,14}=11.21$, $P<0.01$) and biphalin×vagotomy interaction effect ($F_{5,70}=15.22$, $P<0.00001$) 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, and e— $P<0.05$, f— $P<0.01$, g— $P<0.001$, h— $P<0.0001$ versus the respective pre-vagotomy value.

After blockade of μ, κ and δ opioid receptors with an intravenous dose of 1 mg/kg of naloxone hydrochloride in otherwise intact rats, biphalin challenge failed to cause any changes in respiration, mean blood pressure and heart rate (n=8).

Rats subjected to midcervical vagotomy had, inherent in this neurotomy, lower baseline respiratory rates (P<0.00001) and increased tidal volumes (P<0.0001) compared with the intact state, and showed no respiratory responses to administration of biphalin (Table 1).

The results summarized in Table 2 show significant decreases in mean arterial pressure induced by biphalin in the intact rats, initiated in the apnoeic spells and maintained at 10 min. This effect followed a significant early decline in heart rate, lasting 2 min. After bilateral midcervical vagotomy, mean blood pressure had lower baseline values, possibly due to prolonged effects of the previous injection. Blood pressure showed an insignificant trend to decrease, with an onset time 30 s after biphalin challenge. Similarly, heart rate decreased minimally but significantly in the vagotomized rats.

4. Discussion

The present results show that in anaesthetized, neurally intact spontaneously breathing rats the predominant effect of an i.v. injection of biphalin is prompt apnoea, followed by return of ventilation at a persistently slow rate, associated with augmented V_T. This pattern of response differs from that reported by Kamei and Kasuya (1988), namely depression of V_T and respiratory rate. It is of note, however, that they applied biphalin intraperitoneally in the dose range of 0.1–30 mg/kg and measured ventilatory parameters 15 min after the challenge. With the doses used by us (0.3 mg/kg), in our observations the respiratory parameters were approaching baseline values by 15 min post-dosing. Biphalin-induced apnoea occurring in the current experiments paralleled the apnoea observed after systemic injection of DAMGO, morphine and dermorphin in anaesthetized rats (Czapla et al., 2000; Kaczyńska and Szereda-Przestaszewska, 2005; Wojciechowski et al., 2007). Interestingly, central application of enkephalinergic ligands was not reported to evoke apnoea (Hassen et al., 1982; Pazos and Florez, 1983; Rabkin 1991a,b; Chen et al., 1996).

The decrease in breathing rate evoked by biphalin (see Table 1) is consistent with unanimously reported depressive effects of opioid peptides. However, the present study revealed that post-biphalin apnoea is followed by a return of breathing with an increase in V_T, generally observed within 10 min after the challenge. Analysis of the published data does not provide evidence that enkephalinergic ligands or other μ receptor agonists augment tidal volume. However, our findings are consistent with these of Wojciechowski et al. (2007), who reported a similar, although less pronounced effect of the systemic challenge with dermorphin upon V_T. Prompt and persistent post-apnoeic increases in V_T produced by biphalin may be due to activation of rapidly adapting receptors in the lungs that are known to cause hyperpnoea (Sant'Ambrogio and Widdicombe, 2001). The respiratory pattern we report, displaying only one component of hyperpnoea — an increase in V_T, might result from opioid-mediated inhibitory effects upon activation of the sensory nerves within the airways. The transient decrease in V_E, which we observed was then primarily mediated by the decreased respiratory rate.

The respiratory changes produced by i.v. biphalin in the current experiments involve peripheral effects, as indicated by midcervical vagotomy, which in itself increased baseline value of V_T and slowed respiration, preventing all respiratory effects of biphalin (Table 1). Therefore, biphalin-induced activation of μ receptors expressed on pulmonary vagal afferents brought about these changes. This is further supported by the fact, that naloxone acting both centrally and peripherally also effectively blocked the respiratory response, a finding consistent with those of Czapla et al. (2000).

Potent effects of systemically administered opioid peptides on blood pressure and heart rate imply their role in the central and peripheral cardiovascular control. Our report is the first to describe bradycardia and a long-lasting fall in blood pressure produced by biphalin. The same pattern of cardiovascular response but of shorter duration was reported after systemic injections of endomorphins 1 and 2 and DAMGO in anaesthetized rats (Kwok and Dun, 1998; Czapla et al., 2000). In the current experiments stimulation of cardiopulmonary afferents by biphalin resulted in vagally mediated bradycardia and hypotension. Hypotensive effects associated with bradycardia may be secondary to a reduction in cardiac output and/or an effect of baroreceptor's stimulation. Transection of the vagus nerves did not entirely prevent but did reduce post-biphalin bradycardia (see Table 2). This finding is consistent with the cardiac effects of endomorphins (Kwok and Dun, 1998). It is of note that midcervical division of the vagi does not interrupt the superior cardiac branches of the vagal trunk that also may mediate bradycardia. The fall in heart rate may likewise be relayed by a preserved, opioid peptidergic pathway (Búzás and Cox, 1997) from the peripheral baroreceptors, rostral to the level of cervical vagotomy. Our data support this notion, because treatment with naloxone prevented the cardiovascular effects of biphalin.

In summary, the present study is the first to demonstrate that biphalin challenge depresses ventilation, manifested as apnoea, followed by a decrease in respiratory rate and increase in tidal volume. The hypoventilation and concomitant fall in blood pressure are mediated via activation of μ opioid receptors associated with pulmonary vagal afferents, while biphalin-induced bradycardia occurs outside of this pathway.

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