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The effects of alfentanil and selected narcotic analgesics on the rate of action potential discharge of medullary respiratory neurones in anaesthetized rats

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1 The effects of intravenous injections of alfentanil, fentanyl, phenoperidine or morphine on respiratory and peak inspiratory air flow rate, the diaphragm electromyogram (EMG), the activity recorded extracellularly from respiratory neurones located in the ventral respiratory group and the cardiovascular system were examined in anaesthetized rats.

2 Alfentanil produced dose-dependent changes in peripheral and central respiratory parameters, which were prevented by naloxone pretreatment. Minimal effects were produced on the cardiovascular system. The bradypnoea was principally due to a prolongation of the inspiratory phase and was accompanied by a comparable decrease in the peak inspiratory air flow rate.

3 Alfentanil prolonged the discharge duration of inspiratory neurones such that it still maintained a strict phase correlation with the diaphragm EMG, but changes in firing frequency were inconsistent and negligible. The action on expiratory neuronal discharge was analogous to that on inspiratory neuronal discharge but delayed in onset.

4 Hypotension produced by morphine limited the dose used but the respiratory responses to morphine and other selected narcotic analgesics were otherwise similar to that of alfentanil, differing mainly in time-course and magnitude. From the respiratory parameters assessed, the order of duration of effect was morphine > phenoperidine > fentanyl > alfentanil and the relative potencies were 0.1, 0.5, 2.5 and 1 respectively.

5 The selective prolongation of inspiration and the immediate action on inspiratory neurones suggests that systemically administered narcotic analgesics may alter the mechanisms within the central respiratory rhythm generator which determine the cessation of inspiration.

Introduction

Respiratory depression is the commonest problem encountered in the therapeutic use of narcotic analgesics, limiting their usefulness and causing death in overdose (Jaffe & Martin, 1981).

The endogenous opioid peptides [Leu] and [Met] enkephalin (Simantov *et al.*, 1977) and [^{3}H]-diprenorphine binding sites (Atweh & Kuhar, 1977) have a parallel distribution in the rat brain. The brain stem areas with the most dense labelling are the nucleus ambiguus, nucleus tractus solitarius and the parabracheal nuclei, all of which contain neurones thought to subserve a respiratory function (Cohen, 1979). Dynorphin-like immunoreactivity is also found in the nucleus tractus solitarius and the parabracheal nuclei (Zamir *et al.*, 1983). This suggests that endogenous opioid peptides may participate in the mechanisms which determine the output of the central respiratory rhythm generator and that systemically administered morphinomimetics may interfere with the function of respiratory neurones.

Morphine partly depresses respiration by acting on the central chemoreceptors on the ventral medullary surface (Flórez *et al.*, 1968). More recently, respiratory responses have been observed following the local application of morphinomimetics to other brain stem loci (Flórez *et al.*, 1983). Furthermore, the firing rate of medullary respiratory neurones is consistently reduced by the ionotophoretic application of opiates or opioid peptides (Denavit-Saubie *et al.*, 1978; Hewson & Bradley, 1982).

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In the present study, the effects of systemic administration of a number of narcotic analgesics was investigated on the electrical activity of single medullary respiratory neurones. In order that the onset and offset of the respiratory response could be followed, the actions of two very short acting 4-anilopiperidines (alfentanil and fentanyl) and a 4-eq-phenylpiperidine (phenoperidine) were compared with the response to morphine as a standard.

Methods

Peripheral respiratory recording

Wistar rats of either sex weighing 220–305 g were anaesthetized with sodium pentobarbitone (55 mg kg^{-1} i.p.) and supplemented with small doses i.v. as required.

A cannula tied in the trachea and attached in series with a flowhead (type P10, Mercury) enabled respiratory and peak inspiratory air flow rates to be determined by connecting the side arms of the flowhead across a Grass volumetric low pressure transducer (PT5). The amplified signal was displayed on a Grass pen recorder (Model 79D).

The diaphragm electromyogram (EMG) was recorded by a modification of the method of Sears (1964), using a pair of insulated tungsten wires (125 μm diameter) inserted through the intercostal muscles into the costal margin of the diaphragm. The signal was amplified using a Grass P16 preamplifier, displayed on Telequipment (DM63) and Tectronix (502A) oscilloscopes and photographed. The durations of the EMG activity and silence were measured from the film, the inspiratory duration extending from the onset of the EMG to the rapid decline in activity.

All rats were bilaterally vagotomized.

Central respiratory recording

The head was secured in a Précision Cinématographique stereotaxic frame and following occipital craniotomy the cerebellum was removed by suction to expose the dorsal surface of the medulla. Spontaneous electrical activity was recorded extracellularly from medullary respiratory neurones using 5 MΩ tungsten microelectrodes (Clark Electromedical Instruments) with a tip diameter of 1 μm and a Neurolog recording system (Digitimer Ltd). The signal was amplified, filtered (<10 Hz; >40 kHz), monitored through an audioamplifier and displayed on an oscilloscope along with the diaphragm EMG. The spike discharge from a single neurone was differentiated and converted into digital pulses using a window discriminator. The intervals between the pulses recorded during 10 or 15 consecutive respiratory cycles were examined with an interval histogram and an averager module to produce an interspike interval histogram (Gerstein & Kiang, 1960). Each histogram recorded the number of interspike intervals plotted against the time between consecutive spikes.

An IBM computer was used to determine for each neurone, the mean firing frequency (Hz), the duration of each burst of activity (s), the number of spikes per burst and to analyse statistically any drug-induced alteration of the discharge pattern using the Wilcoxon rank-sum test.

The location of respiratory neurones was recorded with reference to the obex and the dorsal surface of the medulla. Only neurones whose discharge showed a perfect phase correlation with the diaphragm EMG without showing any variation in spike amplitude and which had a signal to noise ratio greater than 2.5:1 were included in this study.

An electrocardiogram was monitored (lead III) and the mean systemic blood pressure was recorded from the femoral artery with a Bell and Howell pressure transducer (type 4-422-001) attached to a Grass polygraph.

The body temperature was maintained at 37°C using a homeothermic blanket (Searle Bioscience).

The narcotic analgesic drugs were diluted in 0.154 M sodium chloride solution. Alfentanil was used in doses of 1, 2 or 5 μg kg^{-1}, fentanyl 2 μg kg^{-1}, morphine 50 μg kg^{-1} and phenoperidine 10 μg kg^{-1}. A single dose of the analgesic was injected into the external jugular vein in a volume of 0.4 ml over 2.5 min to minimize the effects on the cardiovascular system. The effects on respiratory parameters were recorded at 2.5, 5, 10, 15 and 20 min from the start of the injection and compared with the average of two control values before and at 2.5 min after a vehicle injection of 0.4 ml sodium cloride solution (0.154 M).

Some rats received naloxone (0.4 mg kg^{-1} i.v.) 12.5 min before 5 μg kg^{-1} alfentanil.

At the end of the experiment rats were artificially ventilated, and after opening the chest, were given succinylcholine (6 mg kg^{-1} i.v.) to paralyse the respiratory muscles. This ensured that the continuing rhythmic neuronal discharge was not due to brain stem movement or proprioreceptor afferent impulses but rather was the activity of a respiratory neurone.

Drugs

The drugs used were: morphine sulphate (Evans Medical), naloxone hydrochloride (Winthrop) and succinylcholine chloride (Calmic). Alfentanil hydrochloride (R3902), fentanyl citrate (sublimaze) and phenoperidine (Operidine) were kindly donated by Janssen Pharmaceuticals.

Analysis of results

To allow a comparison of the findings and to eliminate interindividual variations, the results are expressed as a percentage change (mean ± s.e.mean) from the mean of two control values recorded before and after a vehicle injection. Student's *t* test was used to compare the means, $P < 0.05$ being regarded as significant.

Results

Effect of alfentanil on respiratory and tracheal air flow rates

There was no significant alteration in any of the respiratory parameters measured following the intravenous injection of saline. However, intravenous injection of 1, 2 and 5 $\mu g\,kg^{-1}$ of alfentanil produced a dose-dependent decrease in respiratory rate (Figure 1a). The greatest decreases of 2.1 ± 1.5%, 5.3 ± 1.6% and 16.5 ± 3.6% ($n = 3$, 6 and 10) respectively (from the mean control value of 45.2 ± 2.0 breaths min^{-1}) occurred at 2.5 min after the start of the injection and were significantly different from saline with 2 and 5 $\mu g\,kg^{-1}$ ($P < 0.05$ and $P < 0.01$ respectively). Furthermore, the bradypnoea after 5 $\mu g\,kg^{-1}$ was significantly greater than that produced by 1 $\mu g\,kg^{-1}$ at 2.5 min ($P < 0.01$) and also at 5 min ($P < 0.05$). Irrespective of the dose, the respiratory rate returned to the pre-injection level within 15 min.

Alfentanil also depressed the peak inspiratory air flow rate with a similar time course to the depression of respiratory rate (Figure 1b). The greatest decreases of 7.9 ± 3.0%, 9.1 ± 1.2% and 15.7 ± 2.3% following 1, 2 and 5 $\mu g\,kg^{-1}$ alfentanil ($n = 3$, 6 and 10) respectively (from the mean control peak inspiratory air flow rate of 6.51 ± 0.46 $ml\,s^{-1}$) were also attained at 2.5 min and were significantly different from saline with 2 and 5 $\mu g\,kg^{-1}$ ($P < 0.02$ and $P < 0.001$, respectively). The air flow rate had returned to the pre-injection level by 15 min.

Effect of alfentanil on the diaphragm EMG

Alfentanil caused a rapid but short lasting dose-dependent increase in the duration of the diaphragm contraction which was maximal at 2.5 min after injection (Table 1). At this time the EMG was prolonged from the mean control value of 0.56 ± 0.03 s by 7.2 ± 2.8%, 18.1 ± 3.1% and 51.6 ± 11.0% following 1, 2 and 5 $\mu g\,kg^{-1}$ alfentanil ($n = 3$, 6 and 10) respectively and this was significantly different from saline with 2 and 5 $\mu g\,kg^{-1}$ ($P < 0.01$). The finding that the increases in the ratio of diaphragm contraction to relaxation for these doses of alfentanil (8.5 ± 4.2%, 27.2 ± 5.4% and 44.0 ± 8.7% respectively) were similar to the increases in duration of contraction confirmed the observation that the duration of diaphragm relaxation (Table 1) was not affected by any of the doses of alfentanil. In agreement with the effects on respiratory and peak inspiratory air flow rates the diaphragm EMG activity returned to pre-injection values within 15 min.

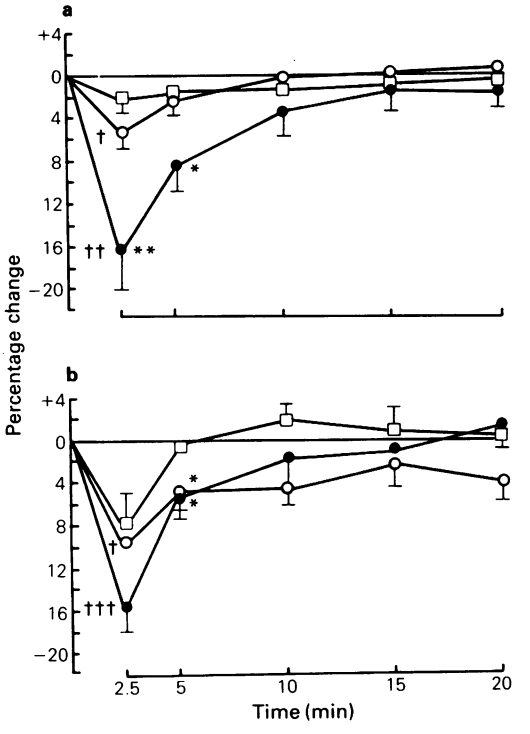

Figure 1 Percentage change in (a) respiratory rate and (b) peak inspiratory air flow rate with reference to time following 1 $\mu g\,kg^{-1}$ (□), $n = 3$; 2 $\mu g\,kg^{-1}$ (○), $n = 6$; or 5 $\mu g\,kg^{-1}$ (●), $n = 10$ alfentanil. Each point represents the mean; vertical lines show s.e.mean. †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$, significantly different from time-matched saline. *$P < 0.05$, **$P < 0.01$, significantly different from 1 $\mu g\,kg^{-1}$ alfentanil at same time (Student's *t* test).

Actions of alfentanil on the cardiovascular system

Respiratory depression was accompanied by minimal changes in the mean systemic arterial blood pressure and heart rate which were unrelated to the dose of alfentanil administered. The greatest change in arterial blood pressure (3.2 ± 5.5% decrease below the mean control value of 88 ± 4 mmHg) occurred 5 min after 5 $\mu g\,kg^{-1}$ alfentanil ($n = 10$), which was 2.5 min after the maximal respiratory effect. The heart rate was unaffected by any dose of alfentanil.

Table 1 The effect of alfentanil on the diaphragm electromyogram

	Dose ($\mu g\,kg^{-1}$)	*2.5 min*	*5 min*	*10 min*	*20 min*
Duration of diaphragm contraction	1	7.2 ± 2.8%	1.9 ± 2.9%	0.5 ± 2.6%	−3.2 ± 3.2%
	2	18.1 ± 3.1%	12.9 ± 3.0%	7.2 ± 1.7%	1.3 ± 1.1%
		*, $P<0.05$	$P<0.05$	NS	NS
	5	51.6 ± 11.0%	27.3 ± 3.9%	[14.0 ± 2.6%]	7.9 ± 1.2%
		*, $P<0.01$	$P<0.01$	$P<0.01$	$P<0.01$
Duration of diaphragm relaxation	1	−2.0 ± 2.3%	−2.7 ± 0.8%	−1.7 ± 2.5%	1.6 ± 3.5%
	2	−6.6 ± 3.8%	−2.4 ± 2.9%	−2.2 ± 1.9%	−3.1 ± 2.6%
		NS	NS	NS	NS
	5	5.8 ± 5.6%	3.0 ± 4.3%	−0.3 ± 4.2%	−2.4 ± 3.4%
		NS	NS	NS	NS

Time course of the change in the duration of diaphragm contraction and the duration of relaxation following 1, 2 or 5 $\mu g\,kg^{-1}$ alfentanil ($n = 3$, 6 and 10 respectively). All values are percentage changes (mean ± s.e.mean) from the mean control values (0.56 ± 0.03 s and 0.86 ± 0.07 s respectively); negative values indicate decreases. The significance of any difference from saline (*$P<0.01$) and from the effect of 1 $\mu g\,kg^{-1}$ alfentanil, according to Student's t test, has been indicated beneath each entry. NS = not significant.

The effect of alfentanil on medullary respiratory neurones

Neuronal activity was recorded from a discrete area 0.3 to 0.5 mm rostral to the obex, 1.2 to 1.6 mm lateral to the midline and between 2.0 and 2.9 mm beneath the dorsal medullary surface. The medullary units were classified as inspiratory or expiratory according to whether their discharge coincided with diaphragm contraction or relaxation (Figure 2). Both types of neurone showed a triphasic discharge pattern similar to that previously described in cats (Kreuter *et al.*,

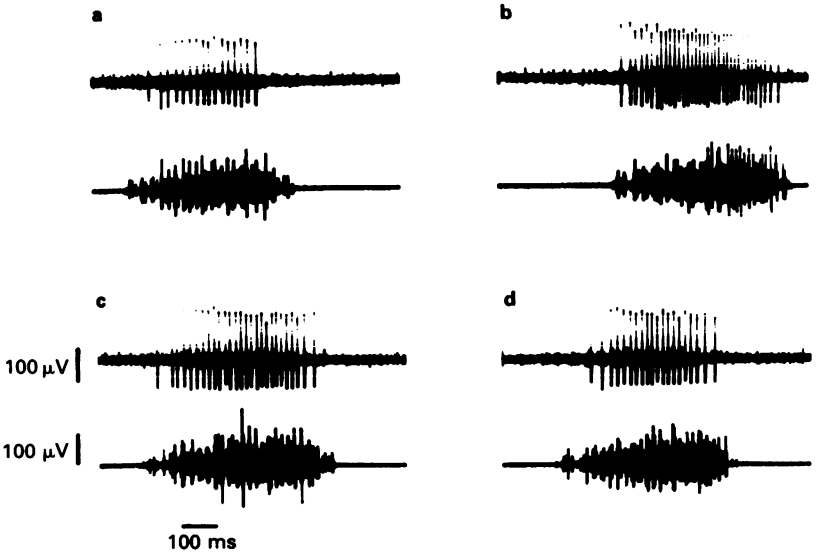

Figure 2 Photographs showing simultaneous recordings of the discharge of a single inspiratory neurone (above) and the diaphragm electromyogram (below) taken (a) before and at (b) 2.5, (c) 5 and (d) 10 min after 2 $\mu g\,kg^{-1}$ alfentanil. The neurone was situated 0.5 mm rostral and 1.3 mm lateral to the obex and 2.78 mm beneath the floor of the fourth ventricle.

1977). Inspiratory or expiratory units which only fired late in inspiration or expiration, or units which had a decremental discharge, were occasionally encountered but were not included in the present study.

Evidence that the discharges recorded came from a respiratory neurone and were not due to respiratory movement was obtained by administering i.v. succinylcholine to artificially ventilated rats. The diaphragm EMG was abolished and although succinylcholine often induced activity in previously silent respiratory neurones, the discharge continued to be phasic, showing no correlation to the ventilator rhythm.

Inspiratory and expiratory neurones were found together in a lamina up to 0.6 mm deep in any particular rat. During the active phase of discharge (excluding the quiescent period), the mean firing frequency of these two types of neurones were 45.3 ± 1.6 Hz and 47.1 ± 3.7 Hz ($n = 24$ and 5) respectively, which were not significantly different. In contrast, however, the mean duration of each burst of activity from inspiratory neurones (0.45 ± 0.04) and the mean number of spikes per burst (21.0 ± 1.5) were significantly less ($P < 0.05$ and $P < 0.02$ respectively) than the corresponding values for expiratory neurones (0.62 ± 0.18 s and 29.4 ± 3.1).

The typical response of an inspiratory neurone to alfentanil is shown in Figure 2. At 2.5 min after administration the neuronal discharge pattern was significantly different ($P < 0.01$; Wilcoxon rank-sum test) from the control pattern. However, at 10 min or more after the injection the discharge pattern for this and all other inspiratory neurones did not differ from the control pattern.

The mean firing frequency of inspiratory neurones (Figure 3a) tended to be elevated by all but the highest drug concentration, but the change was generally small and not dose-related. However, alfentanil produced dose-dependent increases in the number of spikes per burst and in the duration of each burst of inspiratory neuronal activity (Figure 3b and c). In both cases the maximal response occurred 2.5 min after administration; coincident with the greatest change observed in peripheral respiratory activity. At the peak effect, both 2 and 5 $\mu g\,kg^{-1}$ alfentanil significantly increased the number of spikes per burst ($P < 0.05$ and $P < 0.01$) and the duration of each burst ($P < 0.01$ and $P < 0.01$, respectively) of inspiratory neuronal activity when compared with saline.

A comparison of Figure 3c with Table 1 and the visual evidence in Figure 2 shows that, at all doses used, alfentanil produced similar percentage increases in the duration of the inspiratory neuronal and diaphragm EMG activity. This finding and the fact that the linear correlation (according to the method of least squares) between the duration of inspiratory neuronal discharge and the simultaneously recorded diaphragm EMG (Fone, 1984) was still significant when the effect of the highest dose of alfentanil was maximal, shows that the burst of neuronal activity maintains a strict phase correlation with the diaphragm contraction.

Neurones whose discharge was coincident with expiration were examined in five rats which received 5 $\mu g\,kg^{-1}$ alfentanil. The mean firing frequency of respiratory neurones showed little change following alfentanil, irrespective of whether they were inspiratory or expiratory neurones (Figure 4a). In contrast, although alfentanil increased both the number of spikes in, and the duration of, each burst of activity from both inspiratory and expiratory neurones, the time course of the response was clearly different in the two neuronal types (Figure 4b and c). At 2.5 min after alfentanil the number of spikes per burst of activity was reduced by $4.6 \pm 3.5\%$ ($n = 5$) in expiratory but elevated by $39.7 \pm 3.7\%$ ($n = 5$) in inspiratory neurones. This difference was highly significant ($P < 0.01$, according to Student's t test). Subsequently the number of spikes per expiratory neuronal burst increased, reaching $33.7 \pm 17.6\%$ above control

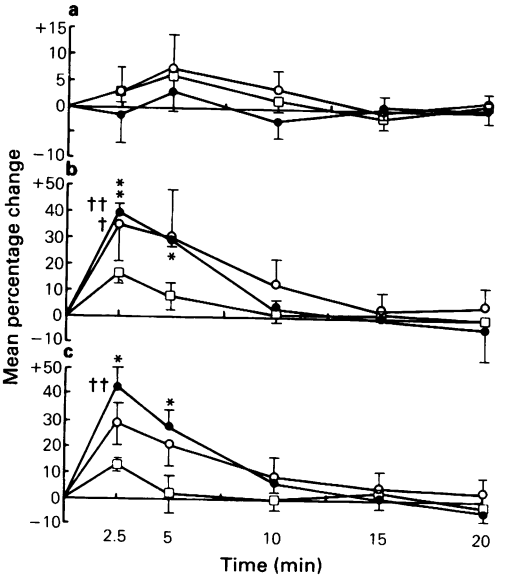

Figure 3 Time course of the change in (a) the mean firing frequency, (b) the number of spikes per burst and (c) the duration of each burst of activity of inspiratory neurones following alfentanil, 1 $\mu g\,kg^{-1}$ (□), $n = 3$; 2 $\mu g\,kg^{-1}$ (○), $n = 5$; or 5 $\mu g\,kg^{-1}$ (●), $n = 5$ expressed as percentage changes from the mean control values (46.7 ± 1.5 Hz, 20.5 ± 1.6 and 0.44 ± 0.03 s, respectively). The significance of any difference from the effect of saline (†$P < 0.05$ and ††$P < 0.01$) or 1 $\mu g\,kg^{-1}$ alfentanil (*$P < 0.05$, **$P < 0.02$) is indicated (Student's t test). Vertical lines show s.e.means.

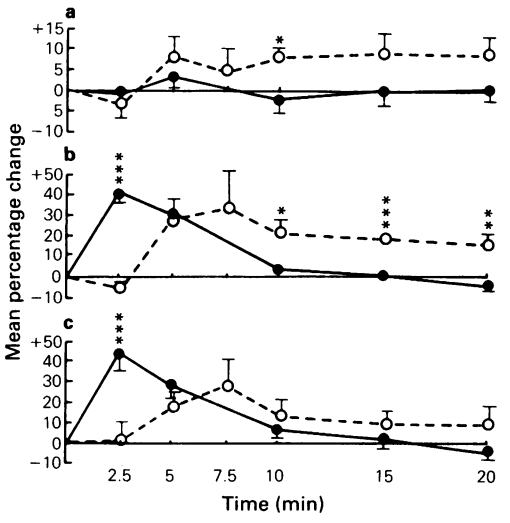

Figure 4 Percentage change in (a) the mean firing frequency, (b) the number of spikes per burst and (c) the duration of each burst of activity from inspiratory (●) $n = 5$ and expiratory (○) $n = 5$ neurones following 5 $\mu g\,kg^{-1}$ alfentanil. Significant differences between the changes in inspiratory and expiratory neuronal activity are indicated: $^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$ according to Student's t test. Vertical lines show s.e.means.

values 7.5 min after alfentanil administration and remained elevated throughout the study period. By comparison the number of spikes per burst from inspiratory neurones progressively returned towards pre-drug values.

The duration of inspiratory and expiratory neuronal discharge was also affected differently by alfentanil (Figure 4c). The inspiratory neuronal discharge was prolonged by 43.3 ± 7.9% at 2.5 min ($n = 5$), when the effect of 5 $\mu g\,kg^{-1}$ was maximal. This value differed significantly ($P < 0.01$) from that obtained for the effect on expiratory neuronal discharge duration, which was only extended by 1.5 ± 9.1% at the same time but reached a maximum value (27.6 ± 13.6% longer than control values, $n = 5$) at 7.5 min.

The Wilcoxon rank-sum test supported the evidence that alfentanil had discriminative effects on inspiratory and expiratory neurones. Since at 2.5 min after the injection the discharge pattern of all the inspiratory neurones but only one expiratory neurone was distinctive from the control pattern at the 95% confidence level.

An intravenous injection of naloxone (0.4 mg kg^{-1}; $n = 3$) 12.5 min prior to alfentanil slightly raised peak inspiratory air flow and respiratory rates but neither effect was significant. Pretreatment with naloxone prevented all the peripheral and central effects produced by 5 $\mu g\,kg^{-1}$ alfentanil.

A comparison of the effects of alfentanil, fentanyl, phenoperidine and morphine

Alfentanil, fentanyl, phenoperidine and morphine all decreased respiratory rate but the time course of each response was different (Figure 5). Bradypnoea was maximal 2.5 min after alfentanil, 5 min after fentanyl, 10 min after phenoperidine and 15 min after morphine; at these times the rates were 16.5 ± 3.6%, 31.6 ± 12.1%, 13.0 ± 2.2% and 7.3 ± 5.2% ($n = 10$, 5, 4 and 4, respectively) below the mean control value (44.1 ± 1.3 breaths min^{-1}). Alfentanil caused the most transient depression of respiratory rate, which returned to control values within 10 min. In contrast, the respiratory rate was still depressed 15 min after fentanyl and 20 min after either phenoperidine or morphine. There was no significant difference in the magnitude of the bradypnoea produced by 5 $\mu g\,kg^{-1}$ alfentanil or 2 $\mu g\,kg^{-1}$ fentanyl. Two and a half minutes after drug administration, alfentanil produced a significantly greater depression of respiratory rate than phenoperidine ($P < 0.02$) or morphine ($P < 0.01$), but a comparison of the peak responses showed there was no significant difference in the magnitude of the bradypnoea produced by any of the drugs. The narcotic analgesics diminished peak inspiratory air flow rate in a similar way to alfentanil, although the effect of morphine was only slight and showed considerable variation. For each drug the time course of these changes paralleled the variation in respiratory rate. When the effect was maximal, the peak inspiratory air flow rate was reduced by

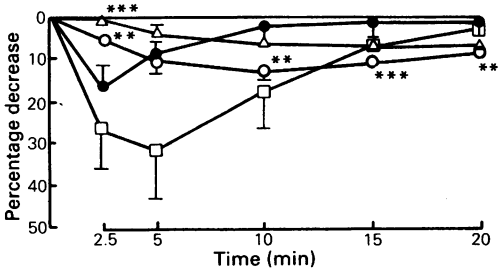

Figure 5 The time course of the percentage change in respiratory rate following administration of either 50 $\mu g\,kg^{-1}$ morphine (Δ), $n = 4$; 10 $\mu g\,kg^{-1}$ phenoperidine (○), $n = 4$; 2 $\mu g\,kg^{-1}$ fentanyl (□), $n = 5$ or 5 $\mu g\,kg^{-1}$ alfentanil (●), $n = 10$. The effect of each drug was compared with that of alfentanil using Student's t test ($^{**}P < 0.02$ and $^{***}P < 0.01$). Vertical lines show s.e.means.

15.7 ± 2.3%, 13.5 ± 3.3%, 6.8 ± 2.7% and 4.9 ± 6.1% below the mean control value of 6.33 ± 0.39 ml s^{-1} by alfentanil (at 2.5 min, $n = 10$), fentanyl (2.5 min, $n = 5$), phenoperidine (10 min, $n = 4$) and morphine (10 min, $n = 4$), respectively. When expressed as a percentage change, the bradypnoea was more pronounced than the decrease in peak inspiratory air flow rate for all drugs except alfentanil.

Although the effect of morphine on the period of diaphragm contraction was small (Figure 6a), a prolongation was consistently observed following narcotic analgesic administration similar to that seen with alfentanil. The maximal response to fentanyl (141.2 ± 92.1% at 5 min, $n = 4$) appeared to be greater than that after alfentanil (51.6 ± 11.0%, 2.5 min, $n = 8$), phenoperidine (38.0 ± 11.4%, 10 min, $n = 4$) or morphine (14.5 ± 10.5%, 15 min, $n = 3$) but this difference was not significant. The rate of onset and offset of the prolongation of diaphragm contraction produced by these drugs was the same as that observed with the effects on other peripheral respiratory parameters measured. As with alfentanil, the bradypnoea produced by fentanyl and phenoperidine was almost entirely due to a prolongation of the inspiratory phase with little effect on the period of diaphragm relaxation. However, the relative ineffectiveness of the dose of morphine used prevented a similar analysis.

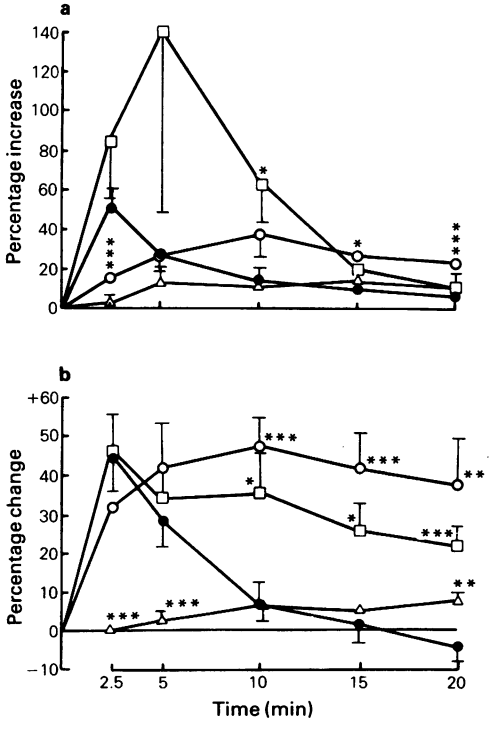

Figure 6 A comparison of the percentage change in (a) the duration of diaphragm contraction and (b) the duration of discharge of medullary inspiratory neurones following 50 μg kg^{-1} morphine (Δ), (a) $n = 3$ and (b) $n = 4$; 10 μg kg^{-1} phenoperidine (○), $n = 4$; or 2 μg kg^{-1} fentanyl (□), $n = 4$ with the effect of 5 μg kg^{-1} alfentanil (●), (a) $n = 8$ and (b) $n = 5$. Statistical differences from the effect of alfentanil are indicated (*$P < 0.05$, **$P < 0.02$ and ***$P < 0.01$, Student's t test). Vertical lines show s.e.means.

Like alfentanil, fentanyl and phenoperidine only produced marginal cardiovascular changes. Although morphine had little effect on heart rate, the mean systemic arterial blood pressure was reduced by more than 20% in two rats, precluding the use of a higher dose of morphine in these experiments.

The narcotic analgesics used in this study never changed the mean firing frequency of individual inspiratory neurones by more than 10%. As noted with alfentanil, all these drugs extended the duration of each burst of inspiratory neuronal activity (Figure 6b). The rate of onset and duration of this action had the same time course as the effect on respiratory rate, peak inspiratory air flow rate and the duration of diaphragm contraction (alfentanil<fentanyl<phenoperidine<morphine). At the peak of the response, alfentanil, fentanyl and phenoperidine caused almost identical increases in the duration of each inspiratory neuronal burst (43.3 ± 7.9% at 2.5 min, 45.4 ± 10.4% at 2.5 min and 47.4 ± 7.8% at 15 min respectively, $n = 5$, 4 and 4) but the effect of morphine was less marked (7.6% ± 2.3% at 20 min, $n = 4$). The number of spikes per burst of activity was also consistently elevated by fentanyl, phenoperidine and morphine, as expected, since the mean neuronal firing frequency was not greatly affected.

The dose of morphine used caused a significant change in the discharge pattern of only one of the four inspiratory neurones examined. For the other opiates the most significant changes from the control pattern of neuronal discharge coincided with the time when the number of spikes in, and the duration of, each burst of activity was increased the most.

The extent of the effects of the selected doses of alfentanil, fentanyl and phenoperidine on both the peripheral and central respiratory parameters were comparable. As preliminary experiments showed that the doses chosen in this study produced a submaximal depression of respiration (unpublished observations), the results suggest that the rank order (and approximate relative potency) in terms of depression of respiration was: fentanyl>alfentanil>phenoperidine (5:2:1). Since the respiratory effects of 50 μg kg^{-1} morphine were not as marked as those of 5 μg kg^{-1} alfentanil it can be concluded that morphine had less than one tenth the potency of the latter.

Discussion

The intravenous injection of alfentanil into anaesthetized rats produced a rapid dose-dependent alteration of both peripheral and central respiratory parameters which was prevented by naloxone pretreatment, indicating that this was mediated by opiate mechanisms. Both the time course and the magnitude of the decrease in respiratory rate were similar to those observed in anaesthetized rabbits (Brown *et al.*, 1980) and conscious human volunteers (Kay & Pleuvry, 1980; Kay & Stephenson, 1981). The bradypnoea was principally due to a prolongation of the period of diaphragm EMG activity without any simultaneous effect on the period of electrical silence, a response frequently encountered when narcotic analgesics are given intravenously to anaesthetized animals (Webber & Peiss, 1975; Denavit-Saubié *et al.*, 1978).

Fentanyl and phenoperidine produced a similar pattern of respiratory depression to that produced by alfentanil, but both had a longer duration of action. In agreement with previous results in rabbits (Brown *et al.*, 1980), fentanyl was found to be 2.5 times more potent than alfentanil at depressing respiration, while phenoperidine was only half as potent as alfentanil.

In contrast to the other narcotic analgesics used in this study, the mild bradypnoea and prolongation of the period of diaphragm contraction produced by morphine were accompanied by small and inconsistent changes in peak inspiratory air flow rate, comparable with previous findings in anaesthetized cats (Ngai, 1961; Flórez *et al.*, 1968). From a comparison of the extent of the respiratory depression it is concluded that alfentanil is at least ten times more potent than morphine.

In agreement with other workers (Sebel *et al.*, 1982; D'Aubioul *et al.*, 1984), the cardiovascular effects following the intravenous injection of alfentanil, fentanyl and phenoperidine were small and in the case of alfentanil, where more than one dose was given, were unrelated to either the dose or the simultaneous respiratory response. Changes in mean systemic arterial blood pressure and heart rate did not precede the respiratory response in a causative fashion but instead occurred simultaneously. In contrast, the marked hypotension produced by morphine, which is probably due to a central action rather than the release of histamine in the rat (Evans *et al.*, 1952), limited the dose which could be used in this study.

To assess the output of the central respiratory rhythm generator, activity was recorded from single spontaneously active neurones in the ventrolateral medulla, whose discharge was phasically linked to that of the diaphragm EMG. These neurones were located in and around the nucleus ambiguus according to the atlas of Pellegrino *et al.* (1979) and are undoubtably part of the ventral respiratory group (Merrill, 1970; Fishman, 1981; Long & Duffin, 1984). The maintenance of rhythmic neuronal discharge after a neuromuscular block with succinylcholine is indicative of activity which has a central origin rather than activity induced by afferent input (Haber *et al.*, 1957), since the preparations were vagotomised and pneumothorax was produced during this procedure.

The incremental type of discharge pattern recorded in the present experiments is characteristic of neurones which have spinal or vagal projections, rather than the activity of interneurones (early-burst inspiratory or early expiratory neurones) which rapidly reach the peak firing frequency at the beginning of the appropriate phase of the respiratory cycle (Mitchell, 1977). The neuronal activity recorded should therefore reflect the output of the central respiratory rhythm generator. In agreement with this argument, the duration and the number of spikes per burst of activity from individual inspiratory neurones was linearly related to the duration of diaphragm contraction, as noted previously (Nelson, 1959). The consistency of the neuronal discharge in each respiratory cycle permitted drug-induced changes in neuronal activity to be examined.

The intravenous injection of narcotic analgesics, in the present study, produced largely inconsistent effects on the firing frequency of individual respiratory neurones, as previously observed in cats (Hassen *et al.*, 1976) following intravenous morphine. However, the duration of each inspiratory neuronal burst and the number of spikes per burst of activity was increased by all the narcotic analgesics used in this study and was elevated in a dose-dependent manner by alfentanil. The duration of each inspiratory neuronal discharge and the duration of the diaphragm contraction were prolonged to a similar extent by alfentanil, indicating that the phasic relation of inspiratory neuronal and diaphragm activity had not been altered. Despite variation in the results with fentanyl this appeared to be true for all the narcotic analgesics used.

Although the intravenous injection of alfentanil produced a quantitatively analogous effect on both inspiratory and expiratory neuronal discharges the expiratory neurones did not appear to be affected until the effects on the inspiratory population were beginning to subside, so the effects on expiratory neurones may have been mediated indirectly. Hassen *et al.* (1976) also found that the intravenous injection of morphine had a differential effect on medullary inspiratory and expiratory neurones although, in this case, the discharge of inspiratory neurones was shortened while that of expiratory neurones was prolonged.

The iontophoretic application of opioid peptides and opiate drugs consistently depresses the firing frequency of medullary respiratory neurones in cats and rats (Denavit-Saubié *et al.*, 1978; Bradley &

Hewson, 1983). Furthermore, Morin-Surun *et al.* (1984b) demonstrated that both inspiratory and expiratory neurones respond in a similar manner to the iontophoretic application of selective μ- and δ-opioid agonists. However, when the same antagonists were given systemically to conscious rats Morin-Surun *et al.* (1984a) noted that inspiratory and expiratory phases were affected differently. It appears that, unlike iontophoresis, the systemic administration of opiates alters the timing of medullary respiratory neuronal activity rather than the firing frequency. This may result from the summation of the effects of systemically administered opiates at other locations involved in the control of respiration, which in turn affect the activity of the ventral respiratory group. In support of this idea, respiratory responses have been observed following the local application of opiates to the chemosensitive areas on the ventral medullary surface (Pokorski *et al.*, 1981; Flórez *et al.*, 1982), the area of the pneumotaxic centre in the dorsal pons (Flórez *et al.*, 1983), the nucleus tractus solitarius (Champagnat *et al.*, 1983), the nucleus ambiguus (Hassen *et al.*, 1984) and also the hypothalamus (Faden & Feuerstein, 1983). The finding in this study that narcotic analgesics selectively prolong inspiration suggests that they may be interfering with the intrinsic mechanisms within the dorsal respiratory group which determine the cessation of inspiration (von Euler, 1980).

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