VENTILATORY EFFECTS AND PLASMA CONCENTRATION OF MORPHINE IN MAN

J. R. A. Rigg

SUMMARY

The relationship between the plasma concentration of morphine and morphine-induced changes in ventilation and the ventilatory response to carbon dioxide was studied in 17 healthy adults undergoing elective surgery under general anaesthesia. Each subject was given morphine sulphate 0.15 mg kg⁻¹ i.m.; ventilation (VE), end-tidal PCO₂(PE'CO₂), mixed venous PCO₂(PvCO₂) and ventilatory response to carbon dioxide (ΔVE/ΔPCO₂) were measured before and within 90 min after injection. Mixed venous PCO₂ and ΔVE/ΔPCO₂ were measured by standard rebreathing methods; plasma morphine concentration was measured by radioimmunoassay. Maximum plasma morphine ranged from 30 to 120 ng ml⁻¹, between 4 and 60 min after injection. There was a significant increase in mixed venous PCO₂ (P<0.001), and PE'CO₂ (P<0.01) after morphine while VE decreased insignificantly. Morphine displaced the carbon dioxide response curve to the right (P<0.01) and ΔVE/ΔPCO₂ decreased from 12.3 to 10.0 litre min⁻¹ kPa⁻¹ (P<0.05). The magnitude of changes in VE and ΔVE/ΔPCO₂ were not related to the peak plasma concentration of morphine or to the mean concentration immediately before and after the carbon dioxide response measurement. Plasma concentrations of morphine, under the conditions of the present study, are not an objective indicator of pharmacological activity between one patient and another.

The development of a sensitive, specific radioimmunoassay for morphine in biological fluids has permitted studies of the disposition of the drug in man (Berkowitz et al., 1975; Laitinen et al., 1975). The value of such pharmacokinetic data is greater if they are related to measurement of biological effect. Although morphine has been used widely as an analgesic for many years, measurement of its clinical effect has been a major problem because methods used to assess pain and its relief are imprecise. This problem has been overcome to some extent by measuring side-effects, such as depression of lung ventilation. Despite many studies, there is no clear agreement on which ventilatory variables are the most precise measurements of the effects of an opiate or on how narcotic drugs disturb ventilatory control (Eckenhoff and Oech, 1960; Campbell, Lister and McNicol, 1964; Jennett, 1968; Jennett, Barker and Forrest, 1968; Weil et al., 1975). The present study was designed to assess the relationship between the disposition of morphine after i.m. injection and changes in ventilation (VE) and ventilatory response to carbon dioxide (ΔVE/ΔPCO₂) in patients awaiting elective surgery.

METHODS

Ventilation, mixed venous PCO₂ (PvCO₂) and ΔVE/ΔPCO₂ were measured before and within 90 min after morphine 0.15 mg kg⁻¹ i.m. given into the right gluteal region of adult patients (American Society of Anesthesiologists physical status I or II) undergoing elective surgery under general anaesthesia. The plasma concentration of morphine was measured by radioimmunoassay in blood samples taken at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 90, 120, 150, 180, and 240 min after injection.

Seventeen surgical patients were studied, six males and 11 females (mean age and SEM = 36.3 ± 3.9 yr, range = 21–68; mean vital capacity and SEM = 3.51 ± 0.20 litre, range = 1.8–5.0). Informed consent was obtained from each patient. None gave a history of cardiovascular, respiratory or neurological disease and all had normal lung function as assessed by spirometry. All denied taking narcotic or other analgesic drugs in the 7 days preceding the study.

Ventilation was measured with a Parkinson–Cowan CD4 dry gas meter, which had a coefficient of variation of 2%. Carbon dioxide was measured with a Godart infra-red analyser, with a response time of 0.1 s, and a precision of ±0.1% over the range 0–10%. The gas sampling point was adjacent to the mouth-piece. The analyser was calibrated with three gas mixtures of carbon dioxide in 40% oxygen in nitrogen, analysed

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previous study in a Lloyd-Haldane apparatus. A continuous record of end-tidal $PCO_2 (PE'CO_2)$ and $VE$ was obtained with an Astromed pen recorder. During rebreathing, the gas sampled by the analyser was returned to the distal part of the bag to prevent recirculation of the sampled gas before complete re-mixing of the gas in the bag. The resistance of the rebreathing circuit was 1.0 cm H$_2$O litre$^{-1}$ s at a flow rate of 4 litre s$^{-1}$.

All studies were performed in a quiet room adjacent to the operating room to minimize unnecessary stimulation. Each patient fasted for at least 4 h and emptied his or her bladder within 1 h of beginning an experiment. An i.v. infusion of saline 100–200 ml h$^{-1}$ was established at the beginning of each study and the infusion cannula was used for blood sampling for the measurement of the plasma concentration of morphine. The initial 2 ml drawn at each sampling was discarded to minimize the possibility of error resulting from dilution of the sample by saline.

Before each carbon dioxide response was measured, $VE$ and $PE'CO_2$ were recorded for 2–3 min and $PVCO_2$ was measured by Collier’s rebreathing method (Collier, 1956). This measurement was used for each patient to adjust precisely the initial $PCO_2$ in the rebreathing bag for the ventilatory response to carbon dioxide measurement; the balance of gas in the bag was oxygen. The bag volume was adjusted to approximately 800 ml greater than the patient’s VC, the minimum volume required to prevent the bag emptying during the large inspirations seen at the end of the period of rebreathing. Rebreathing was begun at the end-tidal position and the patient was immediately instructed to take three large breaths to mix the gas in the lungs and the bag and so attain the rapid equilibration between the bag and mixed venous blood. The appearance of a carbon dioxide plateau on the rebreathing record indicated that mixing of gas in the lungs and bag was complete and that an open loop was established between ventilation and $PCO_2$ (Rigg, Rebuck and Campbell, 1974). In all experiments, such equilibration was observed within 20–40 s. Following the first three large breaths, a spontaneous pattern of ventilation was resumed, and rebreathing continued for 4 min. Preinjection measurements were obtained 30–60 min before the morphine was given. Patients who were not familiar with a mouth-piece and nose-clip or carbon dioxide rebreathing were allowed to become accustomed to these in a preliminary run.

**Estimation of morphine in plasma**

Plasma concentrations of morphine were estimated by radioimmunoassay. Morphine-specific antisera were raised in New Zealand white rabbits by immunization with morphine-6-succinyl-bovine serum albumin (Wainer et al., 1973). To 50 ml of the patient’s plasma sample (diluted 1:5 with saline) 50 ml of 1(n)-3H-morphine (28 Ci mmol$^{-1}$, 5000 ct min$^{-1}$, New England Nuclear) and 100 ml of antiserum (1:4000 dilution) were added. Samples were incubated overnight at 4°C. Five hundred microlitres each of saline and charcoal solution in saline were added to each tube. After centrifugation, the supernatant fluid was transferred to counting vials and mixed with toluene-based scintillation fluid for counting for 4 min in a beta scintillation counter (Phillips’ liquid scintillation analyser). A standard curve was obtained for each assay by adding known amounts of morphine to the tubes, in place of the unknown samples, employing identical experimental conditions. Each sample was analysed in duplicate.

**Specificity of the method**

The specificity of antisera and relative affinity for morphine, morphine metabolites and other opiate alkaloids have been described previously (Wainer et al., 1973). In particular, morphine is bound by antibody five to six times more efficiently than is the major metabolite, morphine glucuronide (Hill et al., 1975). The concentration of morphine in plasma is greater than the concentration of morphine glucuronide for up to 90 min after an i.m. injection (Berkowitz et al., 1975). Thus, detection of glucuronide is unlikely to introduce a substantial error in the estimation of morphine concentration in the present study.

**Data analysis**

The slopes of the ventilatory responses to carbon dioxide were determined by least squares regression, according to the procedure of Read (1967), in which ventilation is averaged over successive 30-s intervals and plotted against $PCO_2$ measured at the mid-point of each interval. Using this procedure, correlation coefficients for the linear regressions (mean and SEM) were: before injection, 0.94 ± 0.015; after morphine, 0.89 ± 0.030. To compare the position of response curves before and after morphine, two values of $PCO_2$ during rebreathing were chosen for each patient. These represented the highest (H) and the lowest (L) $PCO_2$ values, measured by the method described above, common to the two responses. Ventilations observed at these $PCO_2$ values were used to measure the position of the response curve. This procedure was
adopted because different patients rebreathed over widely differing carbon dioxide pressures, as a consequence of widely differing \( P_{\text{CO}_2} \) values between patients, both before and after morphine. Strict criteria for \( P_{\text{CO}_2} \) measurements were observed (Rigg, Rebuck and Campbell, 1974) and all measurements in the present study were within the wide range reported by Smedstad-Sealey, Rebuck and Campbell (1975) in their study of 202 normal subjects. The \( V_H \) and \( V_L \) ventilations (\( V_{E_H} \) and \( V_{E_L} \)) represented actual measurements during rebreathing. The use of these variables to estimate the position of the response curve eliminated errors as a result of extrapolation that would result if a single \( P_{\text{CO}_2} \) value was chosen to express the position of the response curve for all the patients.

The mean plasma concentration of morphine was computed for different sampling times after injection and the results were plotted semi-logarithmically. The elimination of morphine from plasma, following attainment of maximum plasma concentration (\( C_{\text{p,max}} \)), was assumed to be a single exponential function. Rate constants for elimination and half-lives were computed for each individual and mean values calculated.

The interrelationships of changes in ventilatory measurements after morphine and plasma morphine concentration were examined by plotting morphine-induced changes of \( P_{\text{CO}_2} \), \( \Delta V_{E}/\Delta P_{\text{CO}_2} \), \( V_L \) and \( V_{E_H} \) against maximum plasma morphine concentration and the mean of morphine concentrations measured immediately before and after the carbon dioxide response obtained after morphine.

The results were analysed using Student's \( t \) test and least squares regression analysis.

### RESULTS

The maximum plasma concentrations of morphine ranged from 30 to 120 ng ml\(^{-1}\) (mean and SEM: 62.4 ± 6.2), occurring between 4 and 60 min after injection (25.2 ± 3.5). The mean disposition is shown in figure 1. The rate constant for decay (\( k \)) of plasma concentration after \( C_{\text{p,max}} \) was 5.6 ± 0.5 ng min\(^{-1}\), corresponding to a half-life of 124 min (\( t_1/2 = 0.693/k \)).

\( P_{\text{CO}_2} \) \( (P<0.001) \) and \( P_{\text{E',CO}_2} \) \( (P<0.01) \) breathing air, increased after morphine. Minute ventilation, \( V_T \) and \( f \) decreased slightly, but these changes were not significant (table I; fig. 2). The response curve was displaced to the right (\( V_{E_L} \), \( V_{E_H} \); \( P<0.001 \)), and the mean slope decreased from 12.3 to 10.0 litre min\(^{-1}\) kPa\(^{-1}\) \((P<0.05); \) table II; figs 2, 3). Typical effects of morphine on ventilatory responses are shown in figure 4.

#### TABLE I. Mean ventilation (\( \dot{V}_E \) ) tidal volume (\( V_T \) ), breathing frequency (\( f \) ), end-tidal (\( P_{\text{E',CO}_2} \) ) and mixed venous carbon dioxide (\( P_{\text{CO}_2} \) ) before and after morphine (\( n = 17 \))

<table>
<thead>
<tr>
<th></th>
<th>( \dot{V}_E ) (litre min(^{-1}))</th>
<th>( V_T ) (litre)</th>
<th>( f ) (b.p.m.)</th>
<th>( P_{\text{E',CO}_2} ) (kPa)</th>
<th>( P_{\text{CO}_2} ) (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.48</td>
<td>0.73</td>
<td>12.6</td>
<td>4.7</td>
<td>6.1</td>
</tr>
<tr>
<td>SEM</td>
<td>0.43</td>
<td>0.06</td>
<td>0.9</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>After morphine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.76</td>
<td>0.68</td>
<td>12.1</td>
<td>4.9</td>
<td>6.32</td>
</tr>
<tr>
<td>SEM</td>
<td>0.44</td>
<td>0.05</td>
<td>0.8</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>( t )</td>
<td>-1.85</td>
<td>-1.70</td>
<td>-0.91</td>
<td>3.94</td>
<td>6.06</td>
</tr>
<tr>
<td>( P )</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**FIG. 1.** Plasma morphine concentration (mean ± SEM) plotted against time after i.m. injection of 0.15 mg kg\(^{-1}\).
The maximum plasma morphine concentration and the mean of the concentration measured immediately before and after the carbon dioxide response after morphine did not correlate with the magnitude of drug-induced changes of ventilatory variables (fig. 3).

**DISCUSSION**

The results of this study indicate that the absorption of morphine following i.m. injection is variable. Among 17 patients undergoing elective surgery, maximum plasma concentrations of morphine were attained between 4 and 60 min after injection and varied between 30 and 120 ng ml\(^{-1}\). These results are in close agreement with the data of Berkowitz and others (1975), who studied the disposition of morphine following i.m. injection of similar doses in a comparable group. In their study morphine was also measured by radioimmunoassay.

The ventilatory effects of morphine differed. During rebreathing, displacement of the carbon dioxide response curve to the right was the major effect (table II, fig. 2). The findings are in good agreement with data from the literature which suggest consistent changes in position and equivocal or small changes in the slope of the response after morphine (Loeschke et al., 1953; Seed et al., 1958; Bellville, Cohen and Hamilton, 1964; Forrest and Bellville, 1964; Severinghaus and Larson, 1965; Keats and Telford, 1966; Bellville and Fleischli, 1968; Jennett, Barker and Forrest, 1968; Weil et al., 1975).

During air breathing, a small but significant increase of \(P_{\text{CO}_2}\) was observed after morphine, reflecting progressive ventilatory depression and increased carbon dioxide body stores. Ventilation, \(V_t\) and \(f\) measured immediately before carbon dioxide response showed no significant change; this was probably a result of mild hyperventilation immediately before the second carbon dioxide response resulting from the patients' lack of familiarity.
TABLE II. Mean ventilatory response curve position ($\dot{V}_E L$, $\dot{V}_E H$) and slope ($\Delta \dot{V}/\Delta P_{CO_2}$) before and after morphine ($n = 17$). See text for complete explanation of variables

<table>
<thead>
<tr>
<th></th>
<th>$\dot{V}_E L$ (litre min$^{-1}$)</th>
<th>$\dot{V}_E H$ (litre min$^{-1}$)</th>
<th>$\Delta \dot{V}/\Delta P_{CO_2}$ (litre min$^{-1}$ kPa$^{-1}$)</th>
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<tbody>
<tr>
<td>Control</td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>21.62</td>
<td>30.97</td>
<td>12.3</td>
</tr>
<tr>
<td>SEM</td>
<td>1.73</td>
<td>2.48</td>
<td>1.20</td>
</tr>
<tr>
<td>After morphine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>17.47</td>
<td>24.72</td>
<td>10.05</td>
</tr>
<tr>
<td>SEM</td>
<td>1.64</td>
<td>2.26</td>
<td>1.13</td>
</tr>
<tr>
<td>$t$</td>
<td>-5.83</td>
<td>-6.16</td>
<td>-2.32</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

FIG. 4. Ventilatory responses to carbon dioxide in two patients before and after morphine. ($\bullet =$ control; $\circ =$ after morphine.) In each panel, respective $\dot{V}_E L$ and $\dot{V}_E H$ values are indicated together with their corresponding $P_{CO_2}$ values. See text for description of their variables.

The first assumption is that the drug binds reversibly with the tissue receptor; that is, the drug–receptor interaction should obey the law of mass action: a valid assumption for morphine. Second, drug metabolites should not have pharmacological activity and should not be detected by the assay. The major metabolite of morphine, morphine glucuronide, has a minimal pharmacological effect. Morphine glucuronide is detected by the assay, but only one-fifth to one-sixth as efficiently as unconjugated morphine (Hill et al., 1975). Moreover, within the first 90 min after injection, the concentration of morphine is greater than the concentration of morphine glucuronide (Berkowitz et al., 1975). Thus, the latter is unlikely to lead to a substantial error in estimation of the active narcotic. This is supported by the close agreement of our results with those of Berkowitz and others (1975), who demonstrated that their antiserum bound morphine glucuronide at one-eighth of the sensitivity of free morphine binding.

A third assumption is that drug tolerance does not interfere with the plasma concentration–biological effect relationship. Tolerance is a well-recognized property of morphine, but is generally considered to develop slowly. It is unlikely that acute tolerance developing within 2 h could cause such interference.

A fourth assumption is that the plasma concentration should bear a constant relationship to the active tissue concentration. While this may be true for many drugs in the elimination phase (Levine, 1973), it may not be true for morphine during the first 2 h following an i.m. injection. This may reflect many factors affecting absorption and distribution from the site of injection and distribution to effector sites in the central nervous system. A further explanation of the lack of correlation observed between the plasma
morphine concentration and the ventilatory effects may be the lack of specificity and sensitivity of the ventilatory variables used to quantitate the biological effect of the drug. This possibility is supported by recent physiological studies of mental influences on the ventilatory response to carbon dioxide (Rigg et al., 1977) and of pharmacological studies of effects on the ventilatory response to carbon dioxide in volunteers (Jennett, Barker and Forrest, 1968; Rigg and Goldsmith, 1976). It is possible that ventilatory variables do not have the sensitivity or precision required to quantify the biological effect under the conditions of the present study. An alternative experimental design, such as one utilizing a constant i.v. infusion to obtain a constant plasma drug concentration, may enable the demonstration of a quantitative relationship between ventilatory effects of narcotics and their plasma concentrations.

ACKNOWLEDGEMENTS

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REFERENCES


EFFETS VENTILATOIRES ET CONCENTRATION DE MORPHINE DANS LE PLASMA CHEZ L'HOMME

RESUME

La relation qui existe entre les concentrations de morphine dans le plasma et les variations provoquées par la morphine qui se produisent dans la ventilation et la réponse ventilatoire au gaz carbonique a été étudiée sur 17 sujets adultes subissant une intervention chirurgicale à froid sous anesthésie générale. On a administré à chaque sujet une injection intramusculaire de sulfate de morphine à raison de 0,15 mg kg⁻¹; on a mesuré la ventilation (Vₑ), le volume courant (Vₑ₀) et la concentration de PCO₂ (PcO₂⁻) veineux mixte et la
PLASMA MORPHINE AND VENTILATORY EFFECTS

Die Beziehung zwischen Plasma MORPHKONZENTRATION und den dadurch bewirkten Belüftungsänderungen, sowie die Belüftungsreaktion auf Kohlendioxid wurde bei 17 gesunden Erwachsenen untersucht, die sich verschiedenen Eingriffen unter allgemeiner Narkose unterzogen. Jeder Patient erhielt intramuskulär 0,15 mg kg⁻¹ Morphiumsulfat; Belüftung (VE), Ausatmungsvolumen Pco₂ (Pe'co₂), venöses Gemisch Pco₂ (Pv'co₂) und Belüftungsreaktion auf Kohlendioxid (ΔVe/ΔPco₂) wurden vor und innerhalb von 90 min nach der Injektion gemessen. Die beiden letztgenannten Werte wurden durch normale Wiedereinatmungswerte gemessen; die Plasma MORPHKONZENTRATION durch Radioimmunotest. Maximale MORPHKONZENTRATIONEN bewegten sich von 30 bis 120 ng ml⁻¹ zwischen 4 und 60 min nach der Injektion. Es gab einen wesentlichen Anstieg von Pco₂ (P < 0,001) und von Pe'co₂ (P < 0,01) nach Morphium, während Ve unwesentlich absank. Die Kohlendioxid-Reaktionskurve wurde durch Morphium nach rechts verschoben (P < 0,01), und die Belüftungsreaktion auf Kohlendioxid sank von 12,3 auf 10,0 liter min⁻¹ kPa⁻¹ (P < 0,05). Die Große der Veränderungen von Ve und von ΔVe/ΔPco₂ hing nicht mit der Spitzenplasmakonzentration von Morphium oder mit der mittleren Konzentration unmittelbar vor und nach der Messung der Kohlendioxidreaktion zusammen. Die Plasmakonzentrationen von Morphium stellen unter den Bedingungen der gegenwärtigen Untersuchung keine indikative Indikation der pharmakologischen Aktivität zwischen Patienten dar.

SUMARIO
Se estudió la relación entre la concentración de morfina en la plasma y los cambios inducidos por morfina en la ventilación y respuesta ventilatoria al dióxido de carbono en 17 adultos saludables sometidos a cirugía electiva bajo anestesia general. A cada sujeto se le suministró sulfato de morfina 0,15 mg kg⁻¹ intramuscularmente; se midió la ventilación (Ve), Pco₂ (Pe'co₂) de final mareal, Pco₂ (Pv'co₂) venoso mezclado y la respuesta ventilatoria al dióxido de carbono (ΔVe/ΔPco₂) antes y al cabo de 90 min después de la inyección. El Pco₂ venoso mezclado y el ΔVe/ΔPco₂ fueron medidos por métodos de reinspiración normal; la concentración de morfina en la plasma fue medida mediante una radioinmunoprueba. El contenido máximo de morfina en la plasma varió entre 30 y 120 ng ml⁻¹, entre 4 y 60 min después de la inyección. Se produjo un aumento significativo en el Pco₂ (P < 0,001) venoso mezclado, y Pe'co₂ (P < 0,01) después de la morfina, mientras que el Ve disminuyó en forma insignificante. La morfina desplazó la curva de la respuesta del dióxido de carbono hacia la derecha (P < 0,01) y el ΔVe/ΔPco₂ disminuyó de 12,3 a 10,0 litros min⁻¹ kPa⁻¹ (P < 0,05). La magnitud de los cambios en Ve y ΔVe/ΔPco₂ no se relacionó a la concentración máxima de morfina en la plasma ni a la concentración media inmediatamente antes y después de medirse la respuesta del dióxido de carbono. Las concentraciones de morfina en la plasma, bajo las condiciones del estudio actual, no constituyen un indicador objetivo de la actividad farmacológica entre un paciente y otro.