Pharmacokinetics of Morphine:

Concentrations in the Serum and Brain of the Dog during Hyperventilation

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The disposition of morphine in the serum and in the cerebral cortex during normocarbia and hypocarbia was studied in dogs. Normocarbia was maintained by controlled ventilation with air (pHₐ, 7.40; PaCO₂, 40 torr) and by controlled hyperventilation with a gas mixture of CO₂, 3 per cent, O₂, 21 per cent, balance N₂ (pHₐ, 7.40; PaCO₂, 38 torr). Hypocarbia was induced by hyperventilation with air (pHₐ, 7.57; PaCO₂, 20 torr). After achievement of a steady acid-base status, morphine sulfate, 2 mg/kg, was injected intravenously. Thereafter, serial samples of serum and cerebral cortex were taken at intervals for four hours and analyzed for morphine concentrations using radioimmunoassay. In dogs with hypocarbia, serum morphine concentrations 15–240 min following intravenous injections were higher; the serum half-life during the elimination phase remained unchanged, 62–65 min. The apparent volume of distribution, Vₐ, and plasma clearance, Clₐ, of morphine were less during hypocarbia. Morphine concentrations in the cerebral cortex were significantly increased at 15–240 min during hypocarbia. Estimated half-lives of morphine in the cerebral cortex were 5.5 hours during normocarbia, and 8.2 hours during hypocarbia. Maintenance of normocarbia during hyperventilation minimized these changes. These results suggest that during hypocarbia the higher serum morphine concentrations, higher drug distribution coefficient in lipid phase and increased ratio of free base: acid salt of morphine facilitated the penetration of morphine into the brain, in spite of decreased cerebral blood flow. Once in the brain, a greater portion of morphine was probably present in the protonated form in a relatively acidic milieu and thus less able to pass through lipid barriers back to the circulation. (Key words: Acid-base equilibrium; alkalosis, respiratory. Analgesics, narcotic: morphine. Carbon dioxide: hypocarbia. Pharmacokinetics: narcotics, morphine. Ventilation: hyperventilation.)

The pK_a values, partition coefficients, and drug distribution coefficients (apparent partition coefficients, octanol/water) of various narcotics and narcotic antagonists have been reported to be markedly dependent on pH and temperature.1,2 Ainslie et al. have reported that both hypocarbia and hypercarbia changed the concentration and half-life of fentanyl in canine brain.5 We recently reported that respiratory acidosis increased morphine concentrations and prolonged its half-life in the cerebral cortex.8 In this paper, we report our studies of the effects of hyperventilation in the presence or absence of hypocarbia on the concentration–time course of morphine in the serum and in the cerebral cortex in dogs.

Materials and Methods

Mongrel dogs of either sex weighing 9–19 kg were anesthetized with pentobarbital sodium, 30 mg/kg, injected intravenously, and prepared for serial sampling of the blood and the cerebral cortex as described by Finck et al.3 One group of dogs (n = 7) was maintained at normocarbia by ventilation with room air. The minute volume was approximately 200 ml/kg (V_T, 10 ml/kg; f, 20). Mean pHₐ was 7.40 ± 0.01 (SEM), PaCO₂, 40 ± 1 torr, and PaO₂, 87 ± 2 torr. In the second group of dogs (n = 5), the minute volume was doubled, but normocarbia was maintained by using a gas mixture containing CO₂, 3 per cent, O₂, 21 per cent, balance N₂. Mean pHₐ in this group was 7.40 ± 0.01, PaCO₂, 38 ± 1 torr, and PaO₂, 109 ± 3 torr. In the third group of dogs (n = 8), the lungs were hyperventilated with room air with the same minute volume as that of the second group, to give a mean pHₐ of 7.57 ± 0.01, PaCO₂, 20 ± 1 torr, and PaO₂, 105 ± 1 torr.

After 30 min or more of steady acid-base status, morphine sulfate, 2 mg/kg, was injected intravenously over 30 sec. Arterial blood samples were taken 2, 5, 10, 15, 30, 60, 120, 180, 240 min, and cerebral cortex samples 5, 15, 30, 60, 120, 180, 240 min, after the injection. Blood was allowed to clot, and the serum separated by centrifugation and frozen until analyzed. Serial biopsy samples of the cerebral cortex were frozen.

Morphine concentrations in the serum and in the cortical issue were determined in duplicate by radioimmunoassay using rabbit antiserum as described by Spector4 and by Berkowitz et al.6 For the batch of antiserum used for the present study, a 1:1,500 dilution was appropriate.

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Serum morphine concentration profile of individual dogs was fitted by a two-compartment open model using log-linear regression analysis. Serum morphine pharmacokinetic parameters were calculated directly from the coefficients and exponents of the biexponential equation derived from the individual fitting. The brain half-life during 30–240 min was calculated individually from the coefficient of the log-linear regression line.

Comparisons between groups were made by applying Student t tests for unpaired data of serum and brain concentrations, serum morphine pharmacokinetic parameters, and brain morphine half-life.

**Results**

Mean serum morphine concentrations at 15 through 240 min during hypocarbia were significantly higher than those during normocarbia (table 1). Increased pulmonary ventilation, when normocarbia was maintained, did not change serum morphine concentrations significantly. The serum half-lives of morphine during distribution and elimination phases, $T_{1/2a}$ and $T_{1/2b}$, respectively, were similar in all groups. $T_{1/2a}$ values were 4.5 min and $T_{1/2b}$ values varied between 62 and 65 min. The initial volume of distribution, $V_d$, was not altered to a significant extent by hyperventilation. However, the apparent volume of distribution, $V_d$, calculated by the area method, as well as plasma clearance, $Cl_p$, of morphine were significantly decreased by hypocarbia (table 2). $V_d$ and $Cl_p$ in dogs with normocarbic hyperventilation had intermediate values.

The mean morphine concentrations in the cerebral cortex at 15 through 240 min in hypocarbic dogs and at 30 and 60 min in hyperventilated dogs with normocarbic were significantly higher than those of the air-ventilated normocarbic group (fig. 1). In three normocarbic dogs, the morphine concentrations in the cerebral cortex did not decline. In the rest of this group, the mean estimated brain half-life of morphine was 5.5 ± 1.1 hours. In hyperventilated dogs, the mean estimated brain half-lives of morphine were 8.2 ± 0.8 with hypocarbia and 5.1 ± 0.5 hours with normocarbia. The difference between the latter two groups was significant.

**Discussion**

The kinetics of morphine in the serum following intravenous injection into dogs are best fit by a two-compartment open model (fig. 2). The serum morphine elimination half-life is in close agreement to that we previously reported, and averages about an hour. In addition, the absolute values at 2, 5, 10, and

**Table 1. Morphine Concentrations in the Serum Following Intravenous Injection of Morphine Sulfate, 2 mg/kg, under Three Experimental Conditions**

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Normocarbia (n = 7)</th>
<th>Hyperventilation with Normocarbia (n = 5)</th>
<th>Hypocarbia (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.42 ± 0.15</td>
<td>1.68 ± 0.12</td>
<td>1.60 ± 0.15</td>
</tr>
<tr>
<td>5</td>
<td>0.76 ± 0.05</td>
<td>0.95 ± 0.06</td>
<td>0.96 ± 0.07</td>
</tr>
<tr>
<td>10</td>
<td>0.50 ± 0.03</td>
<td>0.59 ± 0.03</td>
<td>0.64 ± 0.06</td>
</tr>
<tr>
<td>15</td>
<td>0.37 ± 0.02</td>
<td>0.43 ± 0.02</td>
<td>0.48 ± 0.04*</td>
</tr>
<tr>
<td>30</td>
<td>0.24 ± 0.02</td>
<td>0.25 ± 0.01</td>
<td>0.32 ± 0.02*†</td>
</tr>
<tr>
<td>60</td>
<td>0.14 ± 0.008</td>
<td>0.167 ± 0.011</td>
<td>0.197 ± 0.013*†</td>
</tr>
<tr>
<td>120</td>
<td>0.067 ± 0.004</td>
<td>0.070 ± 0.011</td>
<td>0.090 ± 0.004*†</td>
</tr>
<tr>
<td>180</td>
<td>0.033 ± 0.002</td>
<td>0.044 ± 0.005</td>
<td>0.054 ± 0.004*†</td>
</tr>
<tr>
<td>240</td>
<td>0.022 ± 0.001</td>
<td>0.028 ± 0.004</td>
<td>0.033 ± 0.003*</td>
</tr>
</tbody>
</table>

Values are means ± SEM; numbers of dogs in parentheses. * † P < 0.05 compared with values during normocarbia and during hyperventilation with normocarbia, respectively.

**Table 2. Pharmacokinetic Values of Morphine in the Serum**

<table>
<thead>
<tr>
<th></th>
<th>Normocarbia (n = 7)</th>
<th>Hyperventilation with Normocarbia (n = 5)</th>
<th>Hypocarbia (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2a}$ (min)</td>
<td>4.5 ± 0.3</td>
<td>4.4 ± 0.2</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td>$T_{1/2b}$ (min)</td>
<td>62 ± 2</td>
<td>65 ± 4</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>$V_d$ (l/kg)</td>
<td>1.01 ± 0.11</td>
<td>0.72 ± 0.02</td>
<td>0.83 ± 0.09</td>
</tr>
<tr>
<td>$V_d$ (l/kg)</td>
<td>4.08 ± 0.25</td>
<td>3.60 ± 0.08</td>
<td>3.23 ± 0.18*</td>
</tr>
<tr>
<td>$Cl_p$ (ml/kg/min)</td>
<td>48.7 ± 3.6</td>
<td>38.7 ± 2.3</td>
<td>34.2 ± 1.6*</td>
</tr>
</tbody>
</table>

Values are means ± SEM; numbers of dogs in parentheses. * P < 0.05 compared with normocarbia.
15 min for the serum and all concentrations in the cerebral cortex are also similar. We did not find any difference in the serum concentrations of morphine in normal dogs between our present and previous study. In this study the morphine concentrations in the sera of normocarbic dogs at one, two and four hours were 0.14, 0.07, and 0.02 µg/ml, respectively. Using similar methods, we previously found morphine values of 0.24, 0.15, and 0.04 µg/ml for the same time intervals.\(^3\)

These differences can best be explained on the basis of different specificities of antisera used.\(^5,6\) In our previous study,\(^3\) the batch of antiserum recognized morphine glucuronide with one-eighth the sensitivity compared with unmetabolized morphine. Morphine glucuronide is the major metabolite of morphine in the dog.\(^10\) The antiserum used in the present study was more specific, in that it recognized the conjugated morphine at one-fortieth the sensitivity compared with unmetabolized morphine. This greater specificity would result in lower serum values of morphine at later time intervals compared with our earlier study. At the initial time intervals (2–15 min) and for morphine concentrations in the cerebral cortex, there would be no difference between these studies, as little morphine glucuronide is expected to be present. The lower serum concentrations of morphine reported here could also explain the larger apparent volume of distribution \((V_d)\), 4.08 l/kg, compared with 2.72 l/kg reported previously.\(^3\)

Intravenously injected drug is initially distributed to and diluted by the central blood volume of the body to establish a certain initial concentration, which then is further decreased by distribution of the drug to the tissues and by elimination. When normovolemia can be assumed, the factors that may decrease cardiac output during mechanical hyperventilation are decreased \(P_{aCO_2}\), increased \(pH\) of the blood, and exaggerated fluctuations in airway pressure, \(i.e.,\) increased mean intrathoracic pressure.\(^11\) Among these, \(P_{aCO_2}\) level is known to play a major role in changing cardiac output.\(^12,13\) In the present study, higher serum concentrations, apparently smaller \(V_t\), and lower \(Cl_p\) of morphine during hypocarbia may implicate a decreased cardiac output.

The higher \(pH\) during alkalosis would result in an increased ratio of free base to acid salt of morphine as predicted by the Henderson-Hasselbalch equation. For the protonated nitrogen of morphine, the postulated \(pK_a\) is 7.93.\(^1\) The solution of the equation gives (free base)/(acid salt) ratios of 1/3.4 and 1/2.3 at the observed \(pH\) values of 7.40 and 7.57, respectively. On the other hand, it has been reported that the increase in \(pH\) is accompanied by a consistent increase in morphine binding to human albumin \(in\ vitro\).\(^14\) An increase of 0.2 \(pH\) unit increases binding from 36 to 39 per cent. If this were the case in the dog, the effect would be a minor decrease in the amount of unbound morphine. Therefore, during hypocarbia presumably more morphine in the serum is available for distribution into the tissue.

Moreover, the drug distribution coefficients (apparent partition coefficients, octanol/water), \(P^*\) for a number of narcotics and narcotic antagonists are sensitive to \(pH\).\(^1\) For morphine sulfate, in particular, at a \(pH\) of 7.40, \(P\) equals 1.42 at 37 C, whereas at a \(pH\) of 7.57, \(P\) is increased to 1.88 (interpolated). This factor would be expected to facilitate the passage of morphine into the brain and other tissues, and to increase \(V_d\). Our finding was, however, a smaller \(V_d\) in the presence of hypocarbia. The reason for this discrepancy is not clear.

\[ **P = \frac{(\text{free base} + \text{acid salt})_{\text{l lipid}}}{(\text{free base} + \text{acid salt})_{\text{water}}} \]

\[ = \frac{\text{(free base)lipid}}{\text{(free base + acid salt)water}} \quad \text{(from Kaufman \textit{et al.}').} \]
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The observed decrease in PaCO₂ from 40 to 20 torr would be expected to decrease cerebral blood flow approximately to half in hypocarbic dogs. The effect of decreased cerebral blood flow would be a diminution of morphine distribution into and egress from the brain. However, our findings suggest that during the distribution phase this effect is probably overcome by increased availability and lipid solubility of morphine in hypocarbic dogs.

Once in the brain, a greater portion of morphine may exist in the protonated form, the acid salt, in relatively acidic milieu. MacMillan and Siesjö estimated the intracellular pH values in the rat brain to be 7.04, 7.10, 7.09, and 7.02 for PaCO₂ values of 38, 26, 15, and 9 torr, respectively, assuming 15 per cent of the cerebral cortex to be extracellular space. Extrapolated to the dog, a lesser portion, approximately 11–13 per cent, of morphine in the cerebral cortex would be available for crossing the lipid barriers back into the circulation. This factor may account for the longer estimated half-life of morphine in the brain than in the serum.

In this study we did not examine the analgesic effect of morphine administered acutely. A reasonable correlation has been reported to exist between the concentrations of morphine in the brain and analgesic activity in mice and rats. Our findings of increased brain morphine concentrations following hyperventilation of dogs could be an explanation of the clinical impression that during anesthesia in man, hyperventilation appears to decrease the requirement for narcotics.

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