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Pharmacokinetics of Morphine:

Effects of Hypercarbia on Serum and Brain Morphine Concentrations in the Dog

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The disposition of morphine in serum and that in the cerebral cortex during normocarbia and hypercarbia were determined in dogs. Normocarbia (pH_a, 7.41; Pa_{CO_2}, 36 torr) was maintained by controlled ventilation. Hypercarbia (pH_a, 7.15; Pa_{CO_2}, 69 torr) was induced by inhalation of 10 per cent CO_2, balance O_2. 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 as long as four hours and analyzed for morphine concentration using radioimmunoassay. Serum morphine concentrations 2 and 5 minutes following intravenous injection were higher in dogs with hypercarbia, although the serum half-life during the elimination phase remained unchanged, 65–67 minutes. The initial volume of distribution, V_i, was smaller during hypercarbia. Also, during hypercarbia morphine concentrations in the cerebral cortex were significantly higher at 15, 60, 120, and 240 minutes than those found during normocarbia. The half-lives of morphine in the cerebral cortex were 4.1 hours during normocarbia and 6.9 hours during hypercarbia. These results suggest that the initial higher drug concentrations in the serum and the increased cerebral blood flow during hypercarbia facilitated the penetrance of morphine into the brain. Once in the brain, a greater proportion of morphine was probably present in the protonated form and thus less able to pass through lipid barriers back into the circulation. (Key words: Acid–base equilibrium, acidosis, respiratory; Analgesics, narcotic, morphine; Carbon dioxide, hypercarbia; Pharmacology, kinetics, morphine.)

THE DISPOSITION of morphine after parenteral administration has been studied in man.[1–4] Of necessity, drug concentrations were measured only in the serum, a compartment separated from the central nervous system by lipid membrane barriers. As morphine is a weak base with relatively low lipid solubility,[5] changes in acid–base status could alter its distribution and elimination. Additionally, acid–base disturbances affect cerebral blood flow,[6] thus the availability of the drug to the brain and its removal from the brain.

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Respiratory acidosis occurs from morphine overdose and unrecognized residual narcosis. We studied the disposition of morphine in the dog, measuring drug concentrations in both serum and the cerebral cortex, and the effect of hypercarbia.

Methods

Mongrel dogs of either sex weighing 10–16 kg were anesthetized with pentobarbital sodium, 30 mg/kg, intravenously. The trachea was intubated with a 10.5-mm cuffed endotracheal tube. Both femoral arteries and the right femoral vein were cannulated. A left craniectomy was performed for subsequent serial sampling of cerebral cortex. The arterial pressure was measured with a Statham P23Db transducer, the expired CO_2 with a Beckman LB-1 infrared gas analyzer. The heart rate, derived from ECG (lead 1) signals, was measured with a cardiotachometer, all continuously recorded on an Offner Dynograph. The rectal temperature was monitored with a mercury thermometer and maintained at 37–38 C, using electric heating pads. Arterial blood gases were measured at intervals (Instrumentation Laboratories, Model 313) during the course of each experiment. Lactated Ringer's solution was infused intravenously at a rate of 10 ml/kg/hr. All animals were ventilated using a Frumin-Lee respirator.

One group of dogs (n = 8) was maintained at normocarbia using room air. Mean pH_a was 7.41 ± 0.01 (SEM), Pa_{CO_2}, 36 ± 1 torr, and Pa_{O_2}, 90 ± 15 torr. In another group of dogs (n = 6) hypercarbia was induced using 10 per cent CO_2 in the inspired mixture. Mean pH_a in this group was 7.15 ± 0.03, Pa_{CO_2}, 69 ± 6 torr, and Pa_{O_2}, 430 ± 26 torr. After a 30-minute equilibration period, control arterial blood and brain samples were obtained. Morphine sulfate, 2 mg/kg, was then injected intravenously over 30 seconds, following which blood and brain samples were taken at 2, 5, 15, 30, 60, 120 and 240 minutes. Blood was allowed to clot, the serum separated by centrifugation and frozen until analyzed. The brain samples (approximately 100 mg each) were blotted dry with pial blood vessels removed and frozen immediately. Hemostasis was secured using oxidized cellulose. The serial biopsy specimens of cortex, which contained gray matter with small but variable amounts of white matter, were taken from areas where

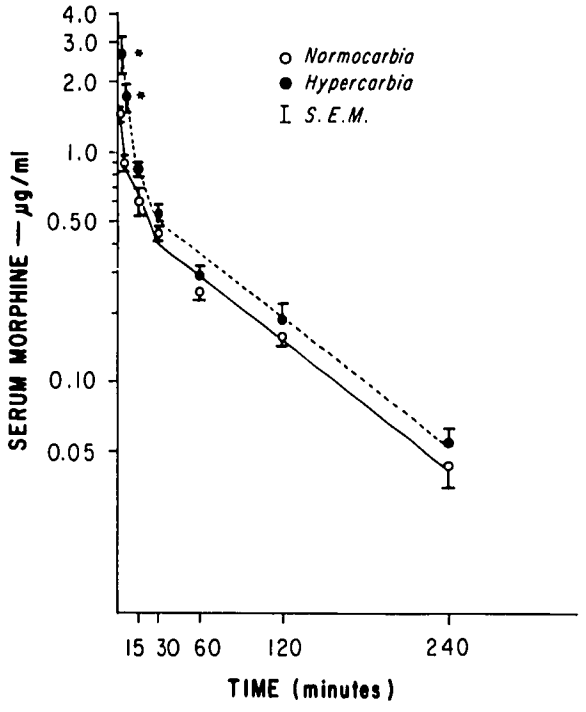

FIG. 1. Serum decrement curves in the dog after morphine sulfate, 2 mg/kg, iv. O —— O normocarbia, ● —— ● hypercarbia. *Significantly different from values during normocarbia, $P < 0.02$.

the circulation had not been grossly disturbed by prior biopsy. Except during biopsy the scalp was closed with skin clips to prevent cooling.

Morphine concentrations in serum and cortical tissue were determined in triplicate by radioimmunoassay using rabbit antiserum.[7] This assay has a sensitivity to quantify picogram amounts of free morphine.[7] The batch of antiserum used recognizes morphine glucuronide but with one-eighth sensitivity compared with free morphine.[4] For the purpose of this study, we did not correct for this systematic error of assay. Brain samples were homogenized in 10 volumes of 0.01 N HCl using a glass homogenizer. After centrifugation the supernatants were diluted 5–40 times for the assay.

Differences between serum and brain morphine concentrations in normocarbic and hypercarbic dogs were compared using Student's t test for unpaired data.

Half-lives were estimated (30 to 240 min in serum and brain) using the slope of linear regression lines calculated after logarithmic conversion of data. The apparent volume of distribution, V_d, was calculated by the "area" method. The initial volume of distribution, V_i, was calculated using the formula $V_i = D/A + B$ where D = administered dose and A and B are the $t = 0$ intercepts for the linear regression lines obtained for the distribution (0–15 min) and elimination phases, respectively.[8] Comparison between normocarbic and hypercarbic groups was made by calculating slopes of linear regression lines, V_d and V_i, for each dog, computing the mean ± SE and using Student's t test for unpaired data.

Results

Mean serum morphine concentrations 2 minutes after intravenous injection were 1.47 ± 0.12 (SE) μg/ml in normocarbic dogs and 2.66 ± 0.56 μg/ml in hypercarbic animals. Concentrations declined rapidly during the first 30 minutes. Serum concentrations at 2 and 5 minutes were significantly higher ($P < .02$) during hypercarbia. The *beta* or elimination-phase half-lives from 30 to 240 minutes were 65 minutes during normocarbia and 67 minutes during hypercarbia, virtually identical, as shown by the close parallelism of the two elimination-phase curves (fig. 1).

The mean peak morphine concentrations in the cortex, achieved 30 minutes after iv injection, were 0.16 ± 0.04 μg/g (wet weight) during normocarbia and 0.20 ± 0.03 μg/g during hypercarbia. The concentration in the brain at 15, 60, 120 and 240 minutes were significantly higher ($P < .05$) during hypercarbia. The average half-life in the brain from 30 to 240 minutes was 6.9 hours during hypercarbia, compared with 4.1 hours during normocarbia (fig. 2). However, the difference in the mean elimination-phase slopes did not reach significance ($P > .05$).

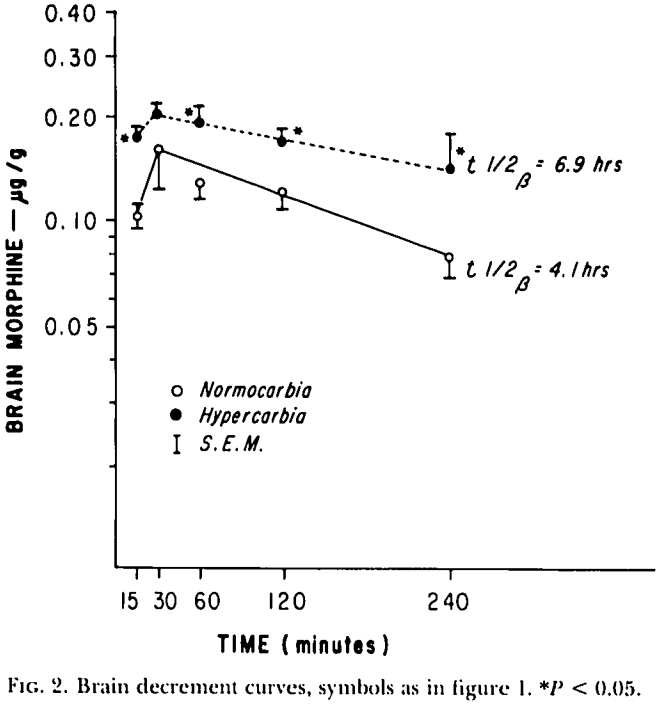

FIG. 2. Brain decrement curves, symbols as in figure 1. *$P < 0.05$.

As shown in figure 3, the elimination-phase curves from the serum and brain compartments intersect rather than decline in parallel.

The apparent volumes of distribution, V_d, were 2.72 ± 0.25 l in normocarbic dogs and 2.04 ± 0.16 l in hypercarbic dogs, though this difference was not significant ($.05 < P < .10$). However, the initial apparent volume of distribution, V_i, was significantly reduced from 1.01 ± 0.08 l in normocarbic dogs to 0.66 ± 0.13 l during hypercarbia ($P < .05$).

Discussion

The morphine concentrations in the serum and the cerebral cortex of the normocarbic dog are similar to those reported by Mulé and Woods,[9] who administered ^{14}C-labeled morphine to dogs and determined radioactive morphine concentrations after extraction. Following subcutaneous injection of 2 mg/kg ^{14}C-morphine into naive dogs, they found plasma levels of approximately 0.29, 0.12, and 0.07 μg/ml at one, two, and four hours, respectively, similar to our findings of 0.24, 0.15 and 0.04 μg/ml in serum after the same intervals. They reported a half-life in plasma of "about one hour," in close agreement with our result of 65 minutes. In brain, these workers also observed a peak concentration in the cortex 35 minutes after subcutaneous injection, which "remained relatively constant over 8 hours." They did not estimate half-life of morphine in the cortex.

The presence of respiratory acidosis caused a reduction in the initial apparent volume of distribution, V_i, by about 40 per cent. The reason for the smaller V_i in the presence of hypercarbia is not clear. Since this is a theoretical compartment without any strict physiologic correlation, one can only speculate as to the possible causes. The effect of a decreased circulating blood volume would be to lower the initial apparent volume of distribution, and such a change cannot be ruled out in the present study. However, a reduction in circulating blood volume to half the control value caused by moderate acidosis seems unlikely.

The effect of acidosis upon the binding of morphine to serum albumin is not known, though it has been reported that increasing pH increases morphine binding to human albumin (an increase of 0.2 pH unit increases binding from 36 to 39 per cent).[10] Were acidosis to cause decreased binding of morphine to serum albumin, the effect would be to decrease the initial apparent volume of distribution. Although the magnitude of changes in plasma protein binding is unknown, it is probably not significant in view of the actual change in pH_a from 7.41 to 7.15.

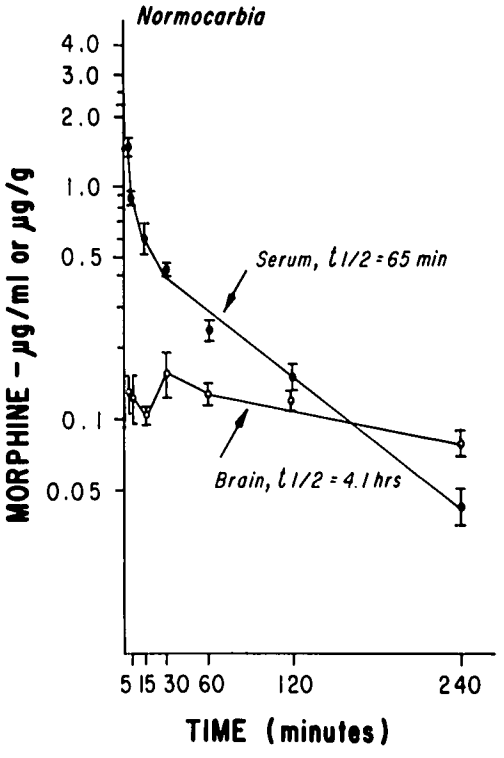

FIG. 3. Serum and brain decrement curves in normocarbic dogs, showing relationship of brain morphine concentrations to those in serum. Note intersection of serum and brain morphine decrement curves at approximately 2.5 hours.

The effect of the lower pH during acidosis would result in an altered ratio of $\frac{\text{(free base)}}{\text{(acid salt)}}$ as predicted by the Henderson-Hasselbalch equation. Using a pK_a of 7.93 for the protonated nitrogen of morphine,[5] the Henderson-Hasselbalch equation at the observed normocarbic pH is:

$$7.41 = 7.93 + \log\frac{\text{(free base)}}{\text{(acid salt)}},$$

solving the equation, $\frac{\text{(free base)}}{\text{(acid salt)}} = \frac{1}{3.3}$.

Using the same equation at the observed hypercarbic pH:

$$7.15 = 7.93 + \log\frac{\text{(free base)}}{\text{(acid salt)}},$$

and upon solving, $\frac{\text{(free base)}}{\text{(acid salt)}} = \frac{1}{6.0}$.

Thus, during hypercarbia, approximately 14 per cent (1/6 + 1) of morphine exists in its free form, compared with 23 per cent (1/3.3 + 1) during normocarbia. As only the free form of morphine passes through biological membranes readily, it would seem

reasonable to assume that during hypercarbia, morphine is distributed to a smaller "volume." This would account for the smaller V_i, apparently smaller V_d, and higher initial serum morphine concentrations in hypercarbic dogs.

The lower *p*H during hypercarbia also lowers the drug distribution coefficient,¶ P, for narcotics. For morphine sulfate in particular, at a *p*H of 7.40, P equals 1.42 at 37 C, whereas at a *p*H of 7.10, P is reduced to 0.80.[5] The decrease in P would result in relatively less free morphine in the lipid phase during hypercarbia. This factor alone would tend to retard the passage of morphine into the brain and other tissues during the distribution phase.

However, the observed increase in Pa_{CO_2} from 36 to 69 torr would be expected to nearly double the cerebral blood flow in hypercarbic dogs.[6] The increased cerebral blood flow, along with the initially higher serum concentrations seen during hypercarbia, would probably overcome the decreased lipid solubility of morphine. The net result was a significantly larger amount of morphine distributed into the brain during hypercarbia, as shown in figure 2.

Once in the brain, which is separated from the circulation by lipid barriers, morphine would egress more slowly from the brain because a greater portion of the drug exists in the protonated form, the acid salt. Less morphine, as free base, is available to cross the lipid barriers back into the circulation.

This might account for the fact that the morphine concentration in the cortex of the hypercarbic animal four hours after administration is almost the same as the peak level achieved in normocarbic animal (fig. 2). The clinical relevance of these findings is not known, but our line of reasoning would suggest that correction of acidosis will speed the efflux of morphine from the brain during the elimination phase, though this has not yet been tested. The results further showed that in the dog the serum concentration of morphine does not reflect that in the brain, as shown by the intersection of the brain and serum elimination-phase curves and the distinctly different half-lives, 65 minutes and 4.1 hours, respectively.

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¶ The drug distribution coefficient $= \frac{(\text{free base + acid salt})_{\text{lipid}}}{(\text{free base + acid salt})_{\text{water}}} \simeq \frac{(\text{free base})_{\text{lipid}}}{(\text{free base + acid salt})_{\text{water}}}$ (from Kaufman *et al.*[5]).