Blood pH and Brain Uptake of $^{14}$C-Morphine

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$^{14}$C-Morphine was injected iv in control awake rats or in rats subjected to metabolic alkalosis or acidosis. Ten minutes later, radioactivity was determined within each of seven brain regions, after correction was made for intravascular tracer. In each region, parenchymal radioactivity was correlated positively and significantly (P < 0.05) with arterial blood pH. Brain radioactivity was twofold to threefold greater in alkalotic rats (mean pH = 7.62) than in acidic rats (mean pH = 7.16). The results are consistent with the pH-partition hypothesis for drug entry into the brain and indicate that morphine uptake can be increased by elevating the fraction of lipid-soluble uncharged morphine base in blood, by means of alkalosis. The observations may account for an exaggerated morphine-induced analgesia in alkalotic patients. (Key words: Acid-base equilibrium; acidosis; alkalosis; Analgesics: morphine. Brain: blood-brain barrier. Pharmacokinetics: morphine.)

Analgesia caused by morphine or its base analogue, meperidine, is increased during alkalosis. For example, surgical patients given meperidine exhibit exaggerated analgesia during respiratory alkalosis,1 whereas rats given morphine have a decreased sensitivity to heat-induced pain when alkalotic.2 The pH dependence of morphine- or meperidine-induced analgesia may depend on pharmacokinetic and pharmacodynamic factors. Brain regions such as the amygdala, periaqueductal gray matter, and thalamus bind opiates specifically.3 Their binding to brain is related to lipophilicity and may be pH dependent for a dissociable base such as morphine.4 On the other hand, according to the pH-partition hypothesis, an alkaline blood pH would be expected to increase brain uptake of morphine by increasing the availability of circulating uncharged base (B), which is about 103 times more lipid soluble and permeant at the blood–brain barrier than the charged form (BH$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^
0.5, 1, 2, 3, 5, and 9 min and were centrifuged. Aliquots of plasma and of a 10-min whole blood sample were placed in vials. Ten minutes after the injection, the rat was decapitated, the brain was removed and the pia-arachnoid, large surface vessels and choroid plexus were separated and discarded. Seven brain regions—hippocampus, hypothalamus, thalamus, midbrain, pons, medulla, and caudate nucleus—were dissected out, placed in tared vials, and weighed. The tissue and whole blood samples were dissolved overnight in 1.5 ml Soluene 350® (Packard Instruments Co., Downers Grove, Illinois), after which a scintillation cocktail was added. Plasma samples were dissolved in 13.5 ml Biofluor® (New England Nuclear, Boston, Massachusetts). Radioactivity (dpm) of plasma, blood, and brain was determined by scintillation spectroscopy (Scintillation Spectroscope No. LS 6800, Beckman Instruments, Inc., Fullerton, California).

Parenchymal brain radioactivity, \( C_{\text{brain}} \text{ dpm} \cdot \text{g}^{-1} \), was calculated by subtracting intravascular brain radioactivity from net measured radioactivity. Intravascular radioactivity was taken as whole blood radioactivity at decapitation, multiplied by 0.02 (estimated intravascular space per gram of brain).9

**Statistics**

Values for brain radioactivity and for the integral of arterial plasma radioactivity were correlated with blood \( pH \). Means in control, acidic, and alkalotic rats were compared by analysis of variance and Bonferroni \( t \) statistics.13 Statistical significance was taken at \( P < 0.05 \).

**Results**

Figure 1 illustrates data from an experiment in which 30 \( \mu \text{Ci} \) [N-methyl-\( ^{14} \text{C} \)]-morphine HCl (i.e., \( ^{14} \text{C} \)-morphine) was injected iv as a bolus in an awake rat, together with 2 mg \( \cdot \text{kg}^{-1} \) unlabeled morphine. The rat first had been infused for 1 h with NH\(_4\)Cl to reduce arterial blood \( pH \) to 7.11. Arterial plasma radioactivity was determined at timed intervals from 0.25 to 9 min following the bolus injection; the integral of plasma radioactivity in this time period equaled \( 1.009 \times 10^8 \) dpm \( \cdot \text{s} \cdot \text{ml}^{-1} \). The rat was decapitated 10 min after injection, and net brain radioactivity was measured at each of seven regions. The table in the figure provides values for \( C_{\text{brain}} \) (parenchymal brain radioactivity), which were calculated from mea-

**Table 1. Brain Radioactivity 10 Minutes after iv Injection of 30 \( \mu \text{Ci} ^{14} \text{C} \)-Morphone in Awake Control, Alkalotic, and Acidotic Rats**

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Arterial Whole Blood pH (7.10 ± 0.01 (8))</th>
<th>( 7.44 ± 0.01 (10) )</th>
<th>( 7.62 ± 0.01 (8) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate nucleus</td>
<td>6.4 ± 0.6†</td>
<td>10.1 ± 1.0†</td>
<td>17.5 ± 4.1</td>
</tr>
<tr>
<td>Thalamus</td>
<td>6.6 ± 0.8†</td>
<td>9.7 ± 0.9†</td>
<td>18.4 ± 4.3</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>11.8 ± 2.3</td>
<td>17.1 ± 1.2</td>
<td>23.2 ± 3.3</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>6.8 ± 0.8†</td>
<td>12.2 ± 1.1†</td>
<td>20.9 ± 4.3</td>
</tr>
<tr>
<td>Midbrain</td>
<td>7.0 ± 0.7†</td>
<td>12.0 ± 1.0†</td>
<td>20.2 ± 4.9</td>
</tr>
<tr>
<td>Pons</td>
<td>6.2 ± 0.8†</td>
<td>10.9 ± 1.2†</td>
<td>18.5 ± 3.7</td>
</tr>
<tr>
<td>Medulla</td>
<td>7.5 ± 0.9†</td>
<td>13.4 ± 1.4†</td>
<td>20.3 ± 3.7†</td>
</tr>
</tbody>
</table>

\[ \int_{t=1}^{t=10} C_{\text{plasma}} \, dt = 1.01 \times 10^8 \text{ dpm sec ml}^{-1} \]

* Mean ± SE (number of animals in column).
† Significantly less than mean at hypothalamus (\( P < 0.05 \)).
‡ Statistically significant correlation (\( P < 0.05 \)).
measured radioactivities after correction for intravascular tracer (see “methods”). For example, $C_{\text{brain}}$ at the hippocampus equaled 8034 dpm g$^{-1}$.

Table 1 presents mean values for $C_{\text{brain}}$ at each of the seven regions that were examined, in acidic animals that were administered NH$_4$Cl (mean blood $pH = 7.16$), in control animals (mean $pH = 7.44$), and in alkalotic animals that were given NaHCO$_3$ (mean $pH = 7.62$). At each region, $C_{\text{brain}}$ was correlated significantly and positively with arterial $pH$. Analysis of variance and Bonferroni $t$ statistics demonstrated, furthermore, that brain radioactivity generally was greater in the hypothalamus than in other regions in control rats ($P < 0.05$). Table 1 also lists the mean arterial plasma integrals of radioactivity, between 0.25 and 9 min after iv $^{14}$C-morphine, at each of the three $pH$ conditions. The integral was not correlated significantly with arterial $pH$.

Figure 2 relates $C_{\text{brain}}$ in a representative brain region, the hippocampus, to arterial $pH$ in each of 26 experiments, and demonstrates a significant positive correlation between the two parameters ($r = 0.56$). Furthermore, it illustrates a more than threefold difference in normalized brain radioactivity between $pH$ 7.1 and 7.7.

**Discussion**

Brain radioactivity at 10 min after iv injection of $^{14}$C-morphine is correlated positively and significantly with arterial blood $pH$ in awake rats. Mean $C_{\text{brain}}$ is twofold to threefold higher at a mean arterial $pH$ of 7.62 than at 7.16. The actual $pH$ during the 10 min of the experiment may have been somewhat lower than the measured preinjection value, however, due to respiratory depression and hypercapnia caused by the injection of unlabeled morphine.

The results agree with observations in anesthetized dogs that morphine uptake by brain is increased during respiratory alkalosis and provide one possible explanation for augmented analgesia in response to meperidine or morphine during alkalosis. These results, and those of Nishitaten et al., are inconsistent with a report that respiratory acidosis increases brain uptake of morphine in the dog. In the latter case, the increased brain uptake was ascribed to hypercapnia, an elevated cerebral blood flow, and increased delivery of morphine to the brain. This interpretation is unlikely, however. The unidirectional transfer constant at the blood–brain barrier for morphine, equal to $k_1 = 5 \times 10^{-4}$ s$^{-1}$, is as low as $k_2$ for sucrose, an agent that enters the brain very slowly. Because $k_1$ for morphine is much less than 0.023 s$^{-1}$, the value for cerebral blood flow in the awake rat, morphine uptake is limited by its diffusion at the blood–brain barrier and is independent of flow.

An elevated brain radioactivity during metabolic alkalosis can be explained in part by the $pH$-partition hypothesis for drug action within the central nervous system. The $pK_a$ of morphine equals 7.93 at 37°C. Therefore, an increase of mean blood $pH$ from 7.16 to 7.62 should increase the percentage of unchanged morphine base (B) in blood from 15% of the net to 33%, or by twofold, approximately equal to the twofold to threefold elevations in brain uptakes noted in this study (table 1). The unchanged base (B) is about 10 times more lipid soluble than is the charged base BH$^+$ and should be proportionately more permeant at the blood–brain barrier.

Greater radioactivity in the hypothalamus than in other brain regions could be due to hypothalamic non-barrier sites with leaky capillaries (e.g., circumventricular organs). These sites would allow charged base into the hypothalamus, independently of $pH$, whereas only uncharged morphine could enter regions with an intact barrier in relation to $pH$. The difference between net radioactivity in the hypothalamus and radioactivity in other brain regions therefore would be decreased by alkalosis, as suggested by table 1.

Within the 10 min after the iv injection of $^{14}$C-
MORPHINE AND BLOOD pH

Morphine, a large fraction of plasma radioactivity is in the form of the radioactive glucuronide. As only free morphine enters the brain, due to the impermeability of the glucuronate at the blood–brain barrier, peripheral pharmacokinetic factors related to pH could modify the availability of free morphine for brain uptake. For example, glucuronidation is accelerated in morphine tolerant animals, but the possible pH dependence of glucuronidation or demethylation is unreported, to our knowledge. The pH gradient between blood and renal tubules also influences renal secretion of unchanged morphine. The initial volume of distribution of morphine depends on blood pH, and the plasma fraction of non-protein-bound drug is elevated by alkalosis.

Cbrain at 10 min after the injection of morphine is at least one tenth of Cplasma at any time during the 10 min (fig. 1). Even allowing for significant glucuronidation of 14C-morphine, the plasma/brain gradient for 14C-morphine is so large during the 10 min that brain radioactivity is due to the unidirectional flux of tracer into brain, with very limited back diffusion from brain. This makes it unlikely that brain pH influences the results.

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References