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

D. S. Schulman, B.A., J. J. Kaufman, Ph.D.,† M. M. Eisenstein, B.A.,‡ S. I. Rapoport, M.D.§*

^{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 *p*H. Brain radioactivity was twofold to threefold greater in alkalotic rats (mean *p*H = 7.62) than in acidotic rats (mean *p*H = 7.16). The results are consistent with the *p*H-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 *p*H 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 *p*H dependent for a dissociable base such as morphine.¶ On the other hand, according to the *p*H-partition hypothesis, an alkaline blood *p*H would be expected to increase brain uptake of morphine by increasing the availability of circulating uncharged base (B), which is about 10^4 times more lipid soluble and permeant at the blood–brain barrier than the charged form (BH^+).[1,4–6] Another possibility is that blood *p*H modifies the rate of morphine glucuronidation or N-demethylation by the liver,[7] but information about this is unavailable. Renal excretion of morphine is influenced by *p*H,[8,9] and alkalosis is reported to increase the percentage of protein-bound morphine in plasma, which normally approximates 35%.[10]

The relevance of *p*H to morphine analgesia is highlighted by evidence in anesthetized dogs that brain uptake of morphine is increased by respiratory alkalosis.[11] We thought it of interest, therefore, to examine this issue during experimental metabolic alkalosis and acidosis and used awake rats for our studies.

Methods

Male adult rats (Osborne–Mendel strain), weighing 250–300 g, were anesthetized with Na pentobarbital (50 $mg \cdot kg^{-1}$, ip), after which indwelling catheters filled with 100 IU Na heparin per milliliter isotonic saline (0.9% [w/v] NaCl) were tied into a femoral artery and vein. Four hours later, when the rats had recovered from anesthesia, blood *p*H was altered in one of two ways. Metabolic alkalosis was produced by an ip injection of $NaHCO_3$ (1.2 $mg \cdot kg^{-1}$ body weight, in isotonic saline). Metabolic acidosis was caused by iv infusion during 60 minutes of 1.2 milliliters of a solution of NH_4Cl, in which the NH_4Cl concentration was adjusted to deliver 535 $mg \cdot kg^{-1}$ body weight. Control rats received ip or iv isotonic saline.

Fifteen minutes after the ip injection of $NaHCO_3$ or of isotonic saline, or at the completion of the 1-h infusion of NH_4Cl or of isotonic saline, 0.25 ml arterial blood were removed for the determination of *p*H (*p*H–Blood Gas Analyzer®, No. 213, Instrumentation Laboratory, Lexington, Massachusetts). The rats then were injected iv with 30 μCi [N-methyl-^{14}C]-morphine HCl (sp. act. = 58 $mCi \cdot mmol^{-1}$; Amersham Corporation, Arlington Heights, Illinois) plus 2 $mg \cdot kg^{-1}$ unlabeled morphine (Applied Science Laboratories, State College, Pennsylvania), both dissolved in 1 ml isotonic saline. Radiotracer purity was ascertained with thin-layer chromatography, using ethyl acetate:methyl alcohol: H_2O:NH_4Cl (85:13.5:1.0:0.5 v/v) as a solvent system and a Whatman® LK5D TLC plate (Bulletin No. 502, Whatman Inc., Clifton, New Jersey). The unlabeled morphine was visualized with ultraviolet light.

After the iv injection of ^{14}C-morphine plus unlabeled morphine, arterial blood samples were removed at 0.25,

* Student Investigator, Laboratory of Neurosciences, National Institute on Aging; presently, Medical Student, Albert Einstein College of Medicine.

† Associate Professor, Department of Anesthesiology, Johns Hopkins University School of Medicine; Principal Research Scientist, Department of Chemistry, Johns Hopkins University.

‡ Student Investigator, Department of Anesthesiology, Johns Hopkins University School of Medicine.

§ Chief, Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health.

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Address reprint requests to Dr. Rapoport: Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, Maryland 20205.

¶ Jacobson AE, Klee WA, Dunn WJ III: A quantitative relationship between antinociceptive activity, opiate receptor affinity and lipophilicity. Eur J Med—Chem Ther 12:49–52, 1977.

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,[12] 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_{brain} dpm · 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).[6]

Statistics

Values for brain radioactivity and for the integral of arterial plasma radioactivity were correlated with blood *p*H. Means in control, acidotic, 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 μCi [N-methyl-^{14}C]-morphine HCl (*i.e.*, ^{14}C-morphine) was injected iv as a bolus in an awake rat, together with 2 mg · kg^{-1} unlabeled morphine. The rat first had been infused for 1 h with NH_4Cl to reduce arterial blood *p*H 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 1.009×10^8 dpm · s · 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_{brain} (parenchymal brain radioactivity), which were calculated from mea-

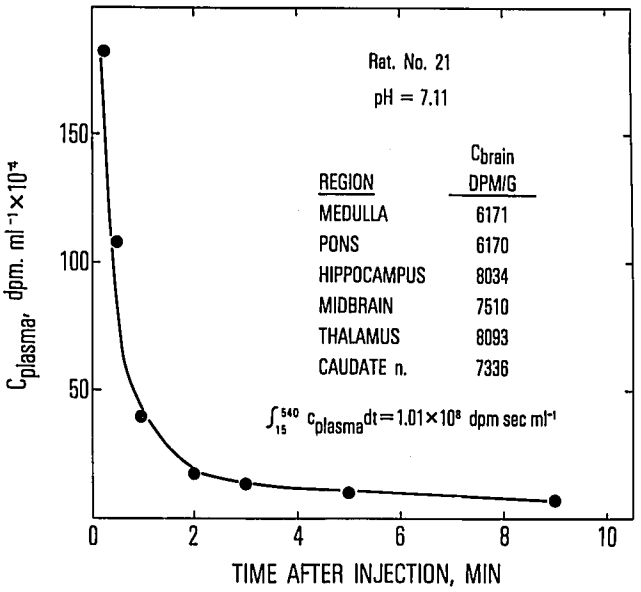

FIG. 1. Plasma and parenchymal brain radioactivities following iv injection of 30 μCi ^{14}C-morphine in a rat. The animal was killed 10 min after injection, and brain radioactivity was corrected for intravascular tracer. Arterial whole blood *p*H = 7.11.

TABLE 1. Brain Radioactivity 10 Minutes after iv Injection of 30 μCi ^{14}C-Morphine in Awake Control, Alkalotic, and Acidotic Rats

Brain Region	Arterial Whole Blood *p*H			Correlation (r) with *p*H
	7.16 ± 0.01 (8)*	7.44 ± 0.01 (10)	7.62 ± 0.01 (8)	
		C_{brain}, dpm · mg^{-1} × 10^{-3}		
Caudate nucleus	6.4 ± 0.6†	10.1 ± 1.0†	17.5 ± 4.1	0.49‡
Thalamus	6.6 ± 0.8†	9.7 ± 0.9†	18.4 ± 4.3	0.48‡
Hypothalamus	11.8 ± 2.3	17.1 ± 1.2	23.2 ± 3.6	0.48‡
Hippocampus	6.8 ± 0.8†	12.2 ± 1.1†	20.9 ± 4.3	0.56‡
Midbrain	7.0 ± 0.7†	12.0 ± 1.0†	20.2 ± 4.9	0.50‡
Pons	6.2 ± 0.8†	10.9 ± 1.2†	18.5 ± 3.7	0.55‡
Medulla	7.5 ± 0.9†	13.4 ± 1.4†	20.3 ± 3.7†	0.57‡
		$\int_{15}^{540} C_{plasma}\,dt$, dpm · s · ml^{-1} × 10^{-8}		
	1.13 ± 0.19	1.41 ± 0.45	1.70 ± 0.38	0.35

Arterial plasma integrals between 0.25 and 9 min after injection also are given in the table. Mean concentrations were compared with the mean at the hypothalamus, by analysis of variance and Bonferroni *t* statistics.

* Mean ± SE (number of animals in column).
† Significantly less than mean at hypothalamus ($P < 0.05$).
‡ Statistically significant correlation ($P < 0.05$).

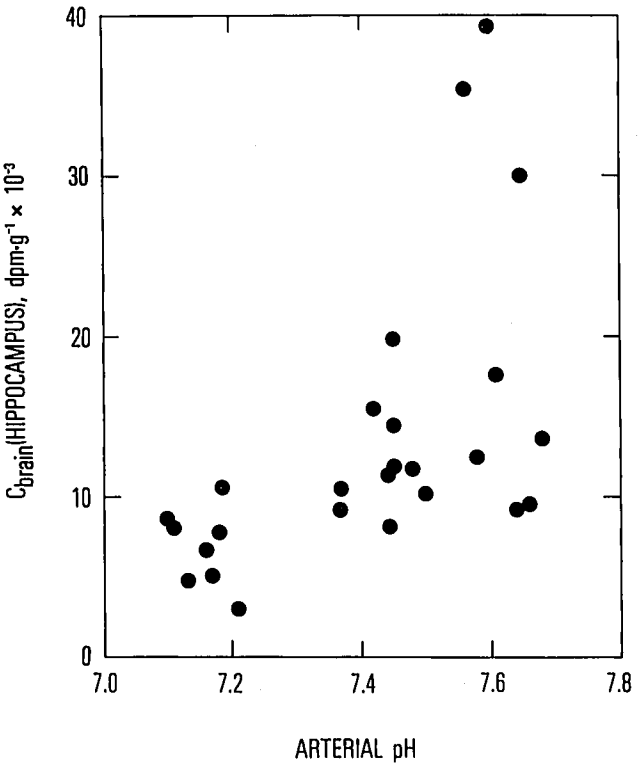

FIG. 2. Relation between C_{brain} at the hippocampus and arterial blood *p*H, in rats injected iv with ^{14}C-morphine. Correlation (r) = 0.56 ($P < 0.05$).

sured radioactivities after correction for intravascular tracer (see "methods"). For example, C_{brain} at the hippocampus equaled 8034 dpm · g^{-1}.

Table 1 presents mean values for C_{brain} at each of the seven regions that were examined, in acidotic animals that were administered NH_4Cl (mean blood *p*H = 7.16), in control animals (mean *p*H = 7.44), and in alkalotic animals that were given $NaHCO_3$ (mean *p*H = 7.62). At each region, C_{brain} was correlated significantly and positively with arterial *p*H. 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 *p*H conditions. The integral was not correlated significantly with arterial *p*H.

Figure 2 relates C_{brain} in a representative brain region, the hippocampus, to arterial *p*H 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 *p*H 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 *p*H in awake rats. Mean C_{brain} is twofold to threefold higher at a mean arterial *p*H of 7.62 than at 7.16. The actual *p*H 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.[14]

The results agree with observations in anesthetized dogs that morphine uptake by brain is increased during respiratory alkalosis[11] and provide one possible explanation for augmented analgesia in response to meperidine or morphine during alkalosis.[1,2,15] These results, and those of Nishitateno *et al.*,[11] are inconsistent with a report that respiratory acidosis increases brain uptake of morphine in the dog.[16] 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},[17] is as low as k_1 for sucrose, an agent that enters the brain very slowly.[5,6,18] Because k_1 for morphine is much less than 0.023 s^{-1}, the value for cerebral blood flow in the awake rat,[19] morphine uptake is limited by its diffusion at the blood–brain barrier and is independent of flow.[5,6]

An elevated brain radioactivity during metabolic alkalosis can be explained in part by the *p*H-partition hypothesis for drug action within the central nervous system.[5] The pK_a of morphine equals 7.93 at 37° C.[20] Therefore, an increase of mean blood *p*H from 7.16 to 7.62 should increase the percentage of uncharged 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 uncharged base (B) is about 10^4 times more lipid soluble than is the charged base BH^+ and should be proportionately more permeant at the blood–brain barrier.[4,21]

Greater radioactivity in the hypothalamus than in other brain regions could be due to hypothalamic nonbarrier sites with leaky capillaries (*e.g.*, circumventricular organs).[5] These sites would allow charged base into the hypothalamus, independently of *p*H, whereas only uncharged morphine could enter regions with an intact barrier in relation to *p*H. 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, a large fraction of plasma radioactivity is in the form of the radioactive glucuronide.[8,14] As only free morphine enters the brain,[7,11,22] due to the impermeability of the glucuronide at the blood–brain barrier, peripheral pharmacokinetic factors related to *p*H could modify the availability of free morphine for brain uptake. For example, glucuronidation is accelerated in morphine tolerant animals,[22] but the possible *p*H dependence of glucuronidation or demethylation is unreported, to our knowledge. The *p*H gradient between blood and renal tubules also influences renal secretion of unchanged morphine.[8,9] The initial volume of distribution of morphine depends on blood *p*H, and the plasma fraction of non-protein-bound drug is elevated by alkalosis.[11,19]

C_{brain} at 10 min after the injection of morphine is at least one tenth of C_{plasma} at any time during the 10 min (fig. 1). Even allowing for significant glucuronidation of ^{14}C-morphine, the plasma/brain gradient for ^{14}C-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.[6,21] This makes it unlikely that brain *p*H influences the results.[22]

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