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Aspects of Morphine Chemistry Important to Persons Working with Cold-blooded Animals, Especially Fish

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The relative amounts of the different forms of morphine, and many other pharmacologic agents, depend on temperature and pH. Some forms are more efficacious because they are uncharged and can penetrate lipid membranes more easily than the charged forms. Persons who administer pharmacologic agents to ectotherms (that is, cold-blooded animals) should consider the effect of temperature on the relative amounts of the different forms of drugs. For example, the fraction of morphine present in the uncharged form is twice as high in a fish or frog at 5 °C as in a mammal at 37 °C. Moreover, because the pH of blood, plasma, and tissues of ectotherms is higher when they are held at lower temperatures, the combined effect of temperature and pH on the speciation of pharmacologic agents also should be considered. In addition, the total solubility of morphine and other pharmacologic agents depends on temperature and pH. The purpose of this overview is to describe how temperature and pH influence the solubility and speciation of morphine.

A review of morphine chemistry is timely for 2 reasons. First is the marked increase in use of fish as a research model in recent years; numbers used now rival those for mice and rats. For example, the Canadian Council on Animal Care reported that in 2003, researchers used 988,784 fish, relative to 789,061 mice and 314,871 rats.[7] In response to the increased use of fish, many organizations have written or are writing animal care guidelines specific to fish.[2,4,6,8] Second, recent reports regarding the use of morphine in fish have attracted much attention in the popular press and gray literature,[5,15,16] but only 1 study investigates morphine pharmacokinetics in fish.[12] These reports have persuaded many of the organizations responsible for writing animal care guidelines to consider the use of analgesics in lower vertebrates. However, persons that use analgesics in lower vertebrates must consider the chemistry of these compounds, especially as influenced by temperature, because most fish, amphibians, and reptiles are ectotherms. Because they cannot generate enough metabolic heat to elevate body temperature much above ambient temperature, their body temperatures are close to that of their environment. These changes in body temperature have important consequences when using morphine in these animals.

The aspects of morphine chemistry of special concern are: solubility of the uncharged free-base form, total solubility, speciation and pK_as, and the effect of temperature on blood pH. We deal with each of these factors in turn and then show how they all must be considered when using lower vertebrates. Our goal is to review the literature on aspects of morphine chemistry that are important to workers who use, study, or treat fish, amphibians, or reptiles. In addition, our remarks may apply to other analgesics, because their properties also will be altered by changes in temperature.

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Morphine Chemistry

Of fundamental importance is the fact that morphine has 2 equilibrium constants and therefore exists in 3 forms in solution: with a charge on the nitrogen ($HOBNH^+$), the uncharged or free-base form (HOBN), and the form lacking the proton on the phenolic hydroxyl group at carbon 3 (^-OBN). At physiologic pH, the $HOBNH^+$ form predominates.

Solubility of the Free-base Form

The relative amount that is in the uncharged free-base form is important because membranes are more permeable to the nonionized form than the ionized form of weak organic bases like morphine. Kaufman and coworkers[9] provide the best estimates of the solubility of the uncharged free-base form in water (Figure 1). There are some estimates that are higher, mostly from papers written in the early 1900s or from studies that did not adjust the pH of the solution to the correct value. For example, Roy and Flynn[14] used a pH value of 8.61, and at that pH and the temperature they used (35 °C), about 77% of morphine is in the uncharged free-base form; whereas the maximum for the uncharged free-base form occurs at a pH of 8.79 at 35 °C. Kaufman and coworkers[9] provide reasonable arguments to support that the values nearest to theirs are most likely to be the most accurate. Regression of those 5 most accurate values (adjusted r^2, 0.99) yields the values in Table 1. Solubility of the uncharged free-base form of morphine in water decreases with a decrease in temperature (Figure 1, Table 1).

Total Solubility—Effect of Temperature and pH

Total solubility is the sum of the solubility of all 3 forms of morphine; it increases with an increase in temperature (Figure 2 A) and decreases with an increase in pH (Figure 2 B). The effect of temperature on the total solubility of morphine hydrochloride has been measured in water, 5% dextrose, and 0.9% saline.[17] That study found no difference between total solubility in water or 5%

Table 1. Solubility of the uncharged free-base form of morphine sulfate (HOBN) in water

Temperature (°C)	Solubility of free base (mg/ml)
5	0.12
10	0.13
15	0.14
20	0.15
25	0.16
30	0.17
35	0.18

Note that the solubility of the free-base form of morphine increases with an increase in temperature.

dextrose, but total solubility was markedly reduced in 0.9% saline relative to water or 5% dextrose. However, the experiments presented in that paper trigger 3 concerns.[17] First, the Methods state that total solubility was measured at 4, 10, 15, 20, 25, 30, 35, 40 °C, but the points on the figure show values at 1, 8, 15, 22, 25, 30, and 40 °C. Therefore some of the points in the figure appear to be plotted incorrectly. Second, the authors state that total solubility of morphine in water is 50 mg/ml at 22 °C and 30 mg/ml at 4 °C, whereas the graph shows 48 mg/ml at 22 °C and 35 mg/ml at 4 °C. We therefore assume that the statements in the results are general in nature. Third, and more troublesome, the authors state that total solubility of morphine in saline is 30 mg/ml at 22 °C and 20 mg/ml at 4 °C, whereas the graph shows 28 mg/ml at 22 °C and 14 mg/ml at 4 °C. This difference at 4 °C (20 versus 14 mg/ml) is substantial and cannot be taken as a generalization from their results. For the purposes of our analysis, we have used the points plotted in the published figure.[17] The effect of temperature alone suggests that the total solubility of morphine in a fish at 5 °C is less than one-half of what it is in a mammal at 37 °C.

The total solubility of morphine hydrochloride is less in saline than in water (Figure 2 A) because of the common-ion effect. The common-ion effect is the reduction in solubility of a sparingly soluble salt by the addition of a soluble salt that has an ion in common with it. Adding any chloride salt to a solution of morphine hydrochloride will reduce its solubility. The forms in solution are in dynamic equilibrium with the solid form:

$$M^*HCl(solid) \leftrightarrow MH^+ (aqueous) + Cl^- (aqueous)$$

and

$$K_{sp} = [MH^+] \times [Cl^-],$$

where K_{sp} is the solubility product of morphine chloride. We can estimate the expected decrease in 0.9% saline used in the study by Vermeire and Remon,[17] who measured the total solubility of morphine hydrochloride and reported a maximum of 82.18 mg/ml at 35 °C. This value is equal to a molar solubility of 0.256 mol/l (molar mass is 320.8 g/mol). Thus K_{sp} can be estimated as $0.256 \times 0.256 = 0.0656$.

For solutions that contain a common ion, the K_{sp} relation can be rearranged so that

$$[MH^+] = K_{sp}/[Cl^-].$$

Clearly, if we add saline and increase $[Cl^-]$, the equilibrium moves to the insoluble form, takes MH^+ out of solution, and its measured solubility decreases. If we add 0.9% saline (0.154 mol/l) then we increase $[Cl^-]$ from 0.256 to 0.256 + 0.154 = 0.41 and predict that $[MH^+]$ will precipitate out and decrease from 0.256 in water to 0.16 in saline. This calculated 37% decrease is close to the 33% solubility decrease previously reported[17] (Figure 2 A). The common-ion effect would not apply to morphine sulfate, because there is no common ion.

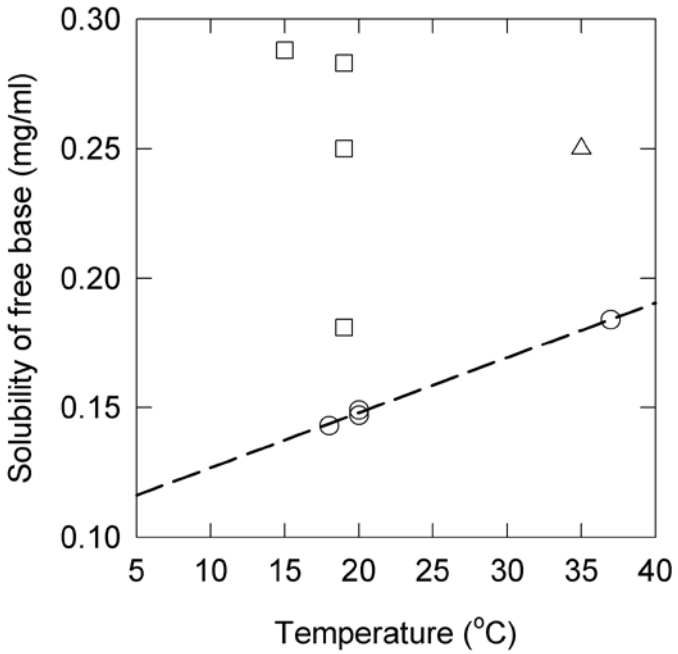

Figure 1. Solubility of the uncharged free-base form of morphine sulfate (HOBN) at high pH increases with an increase in temperature. Open circles are from reference 9 and references therein; the line represents least-squares regression using the most reasonable values. Open squares (reference 9 and references therein) and open triangle[14] are from studies with excessively high values, probably because the pH was not appropriate and therefore some of the measured morphine was ionized and not in the free-base form.

Total solubility also is affected markedly by the pH of the solution—total solubility decreases as the solution is made more alkaline (Figure 2 B). This effect is important because morphine for injection is always acidic and is injected into an animal that invariably has a much higher pH. The change in pH upon injection decreases total solubility by at least 2 orders of magnitude (Figure 2 B).

The combined effect of temperature and pH on the total solubility of morphine has not been reported, but we can combine the temperature and pH data in Figure 2 to estimate the summed effect. This combined effect of temperature and pH is illustrated by example in Figure 3. The total solubility of morphine is less in a fish at 10 °C than a dog at 37 °C in part because of the temperature effect and in part because of the pH effect (as discussed later, the pH of fish blood is higher at lower temperatures).

These results have important implications for some published experiments regarding the use of morphine in fish. In the frequently cited experiments of Sneddon, morphine sulfate in saline was injected at a concentration of 30 mg/ml into fish at 11 °C[15] and 14 °C.[16] The solubility data in Figure 2 indicate that morphine sulfate would precipitate at this concentration, temperature, and at any pH > 5.7, that is, at any physiologic pH.

The discussion presented thus far has focused on the solubility of morphine in water and saline solutions because that is what has been studied experimentally. When administered to mammals, about one-third of morphine is bound to plasma proteins, a proportion which is low relative to other drugs and is unlikely to alter any of our arguments in a substantive way. Moreover, it is important to appreciate that albumin is present in the plasma of some fish but absent in others. For example, none of the sharks or

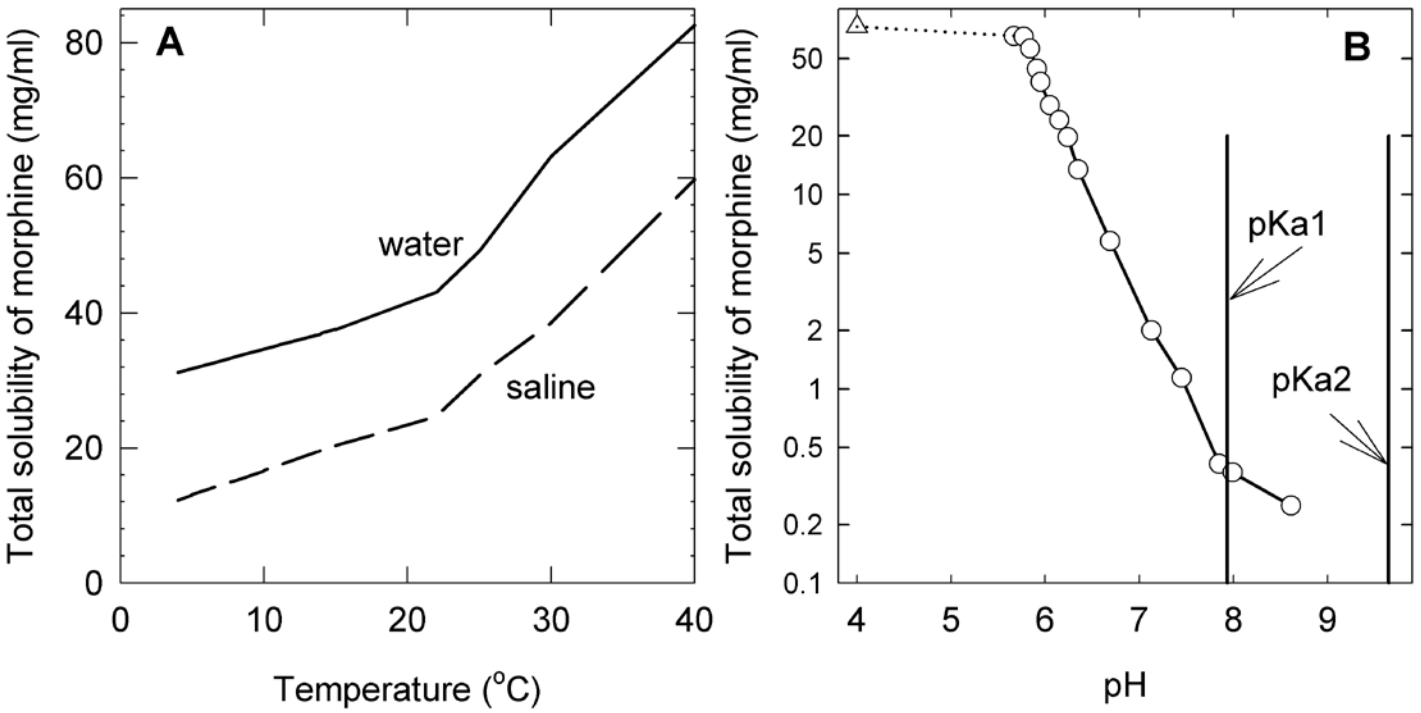

Figure 2. (A) Total solubility of morphine increases with an increase in temperature. The total solubility of morphine hydrochloride reported by Vermeire and Remon[17] is the same in water and 5% dextrose (solid line) but is much greater than the total solubility in 0.9% saline (dashed line) because of the common ion effect. Values plotted have been converted from morphine HCl to morphine by using the ratio of the molar masses (285.33/321.79). The pH was acidic; it varied from 4.9 to 3.8 for morphine in water and from 4.3 to 4.8 in saline; therefore more than 99.9% of the morphine was in the $HOBNH^+$ form at all temperatures. (B) Total solubility of morphine decreases with an increase in pH. Open circles are total solubility values of morphine in water at 35 °C with pH controlled using 100 mM citrate buffer.[14] The decrease in total solubility is more than 2 orders of magnitude (the scale on the *y* axis is log scale). Vertical lines indicate pKa1 and pKa2 of morphine at 35 °C. The single open triangle is for morphine (converted from mg/ml morphine hydrochloride) as reported by Vermeire and Remon.[16]

rays have plasma albumin. Some bony fish have an albumin-like protein in their plasma, and some do not. In so far as we can determine, the protein binding of morphine (or of any other opioid) has not been studied experimentally in any ectotherm. Clearly, research on the topic of binding of morphine and other drugs to plasma proteins is needed.

Speciation of Morphine

Morphine has 2 equilibrium constants (pK_{a1} and pK_{a2}) and therefore exists in 3 forms in solution: with a charge on the nitrogen ($HOBNH^+$, pK_{a1}), the uncharged or free-base form (HOBN, pK_{a2}), and the form lacking the proton on the phenolic hydroxyl group at carbon 3 (^-OBN). At physiologic pH values, $HOBNH^+$ predominates. K_{a1} is the equilibrium constant for

$$HOBNH^+ + H_2O \leftrightarrow HOBN + H_3O^+.$$

K_{a2} is the equilibrium constant for

$$HOBN + H_2O \leftrightarrow {}^-OBN + H_3O^+.$$

Because the pK_as are temperature-dependent, the relative amounts of the 3 forms change with temperature. To ensure that morphine for injection does not precipitate, it is distributed in vials that are acidified so that essentially all of the drug is in the $HOBNH^+$ form. The morphine that we use in our experiments (Sabex, Canada; morphine sulfate injection USP) has a pH of 3.2.

As mentioned, morphine and similar compounds have 2 pK_as, 1 for the proton on the nitrogen and 1 for the phenolic hydrogen. Many papers[9] report only a single value, and that value is not a valid pK_a for either pK_{a1} or pK_{a2} but is "some average of the two."[9] An understanding of the pK_as of analgesics is important

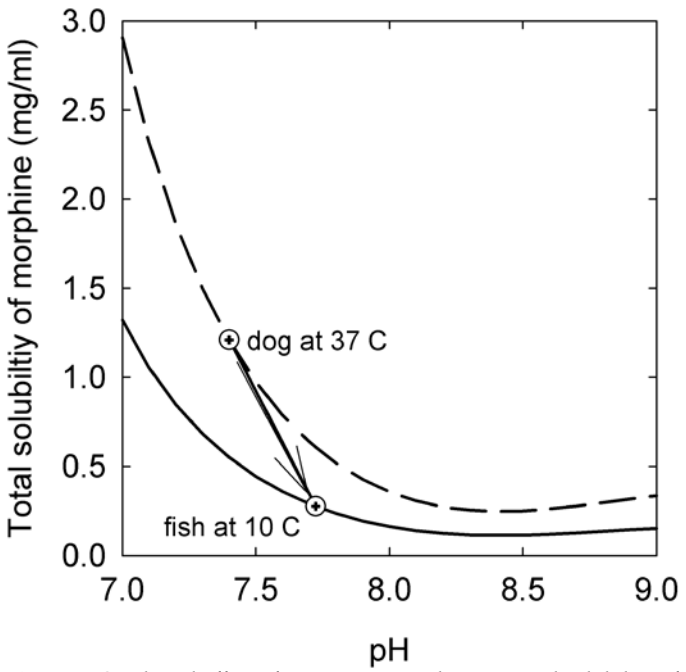

Figure 3. Combined effect of temperature and pH on total solubility of morphine. The temperature effect is illustrated by the 2 lines—the dashed line is total solubility at 37 °C and the solid line is that at 10 °C. The points for a mammal at 37 °C and for a fish at 10 °C are at the normal blood pH values (7.4 for the mammal at 37 °C and 7.7 for a fish at 10 °C). The decrease in total solubility is a combined effect of both temperature and pH.

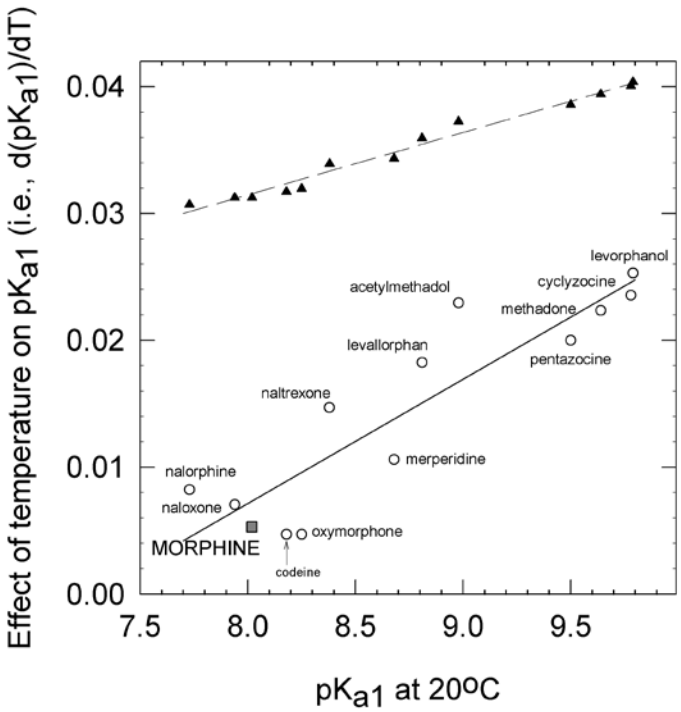

Figure 4. Relation between effect of temperature on pK_{a1} at 20 °C for a variety of analgesics and similar compounds. The effect of temperature on pK_a is defined as the slope of that relation, that is, the change in pK_a per change in temperature (degrees Kelvin), or d (pK_a)/dT. Data is from Table 3 of reference 9 (square, morphine; open circles, other compounds; solid line is least-squares regression line). The dashed line and closed triangles show the theoretical slope calculated using the relation in Albert and Serjeant[1] for the same compounds.

because their efficacy depends in part on their lipid solubility, which, in turn, depends on the pK_as. This is because only the uncharged free-base form of morphine (that is, HOBN) will tend to partition into lipids and cross membranes.

Predicting the effect of temperature on the pK_a of any compound is difficult. In the authority on the topic, Albert and Serjeant[1] give a general equation for the effect of temperature on pK_{a1} for nitrogenous bases that has been used by others to estimate pK_{a1} for morphine.[14]

For compounds with 1 pK_a and for pK_{a1} for compounds with 2 pK_as

$$\frac{-d(pK_{a1})}{dT} = \frac{pK_{a1} - 0.9}{T},$$

where T is temperature in degrees Kelvin, and for pK_{a2} for compounds with 2 pK_as

$$\frac{-d(pK_{a2})}{dT} = \frac{pK_{a2}}{T}$$

These theoretical values are plotted in Figure 4 with the slopes for a variety of analgesics and similar compounds by using the data summarized in Kaufman and coworkers (from Table 3 of reference 9). The data illustrate the point that, in general, the effect of temperature increases with pK_a but that the theoretical calculation overestimates the influence of temperature for opioids and similar compounds. In particular, the effect of temperature on morphine is greatly overestimated using the theoretical estimate from Albert and Serjeant[1] and explains why some pK_a values in the literature (for example, reference 14) are incorrect.

From the discussion presented, we know that both pK_{a1} and pK_{a2} decrease with an increase in temperature and that some of the data in the literature is incorrect. Therefore we have reviewed the literature and summarized it in Figure 5. Reasonable arguments support deduction of estimates.[9] Figure 5 also shows that the theoretical calculation used by some authors overestimates the effect of temperature and thus influences their estimates. Estimates of pK_{a1} and pK_{a2} of morphine at a variety of temperatures that may be useful to those researchers doing experiments on ectotherms are presented in Table 2.

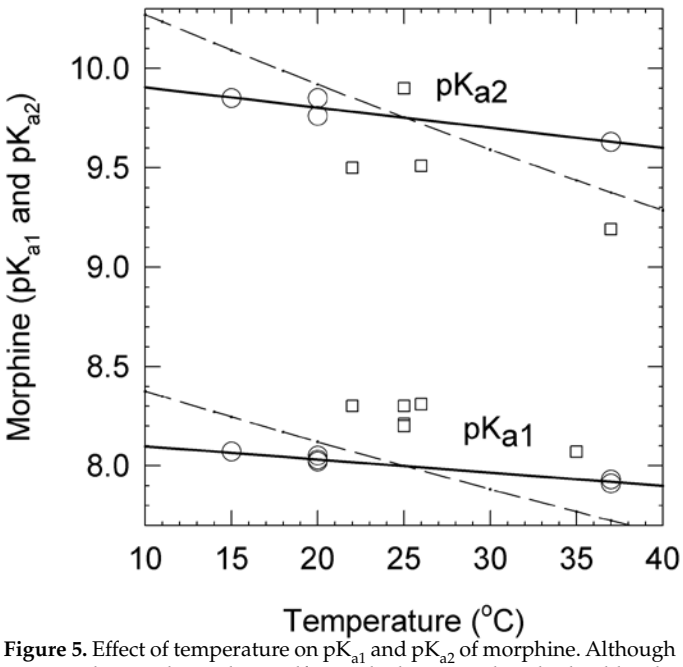

Figure 5. Effect of temperature on pK_{a1} and pK_{a2} of morphine. Although some studies used morphine sulfate and others morphine hydrochloride, this difference should not alter the estimate. Open squares are published values not included in the regression; open circles and solid line are values used in the regression that are the best estimates in our view. Dashed lines represent the theoretical effect of temperature calculated using the equations in Albert and Serjeant.[1]

Table 2. Estimates of pK_{a1} and pK_{a2} for morphine in water that may be of use to researchers using morphine in ectotherms

Temperature (°C)	pK_{a1}	pK_{a2}
5	8.13	9.95
10	8.10	9.90
11	8.09	9.89
14	8.07	9.86
15	8.07	9.85
20	8.03	9.80
25	8.00	9.75
30	7.97	9.70
35	7.93	9.65
37	7.92	9.63

Effect of pH and Temperature on the Speciation of Morphine

As mentioned earlier, morphine in solution exists in 3 molecular forms, and the relative contribution of the forms depends on solution pH and the pK_a values, which in turn depend on temperature. At any particular temperature, the fractional contribu-

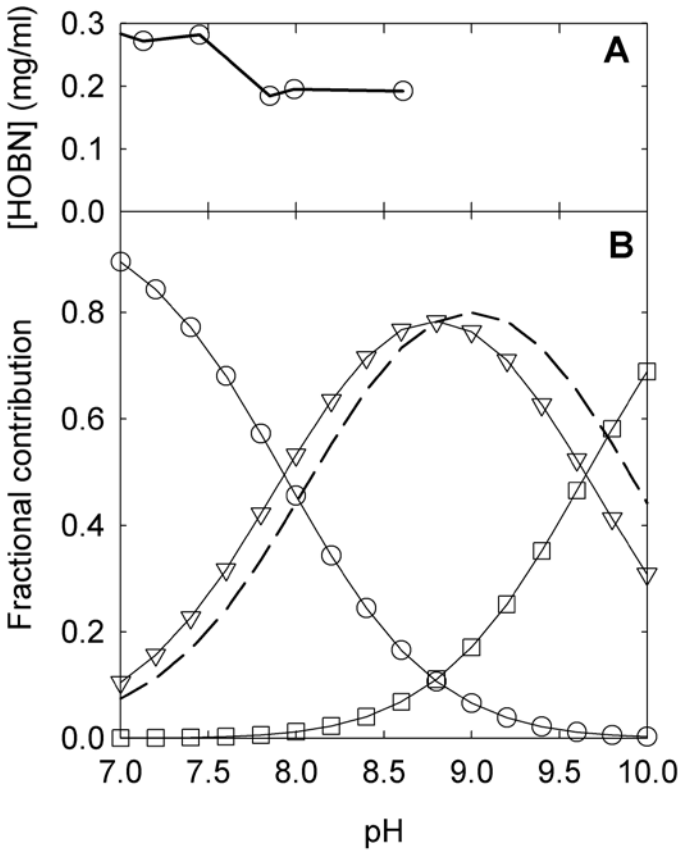

Figure 6. (A) Change in concentration of the uncharged form (HOBN) at 35 °C[14] with a change in pH. Concentration calculated from total solubility in Figure 2 B[14] and the fractional contribution in Figure 8. (B) The fractional contribution of the 3 molecular forms of morphine depends on the pH. Values at 35 °C are shown with the solid lines and symbols. The dashed line shows the fractional contribution of the uncharged form (HOBN) at 10 °C. At 35 °C, the fractional contribution of the uncharged form (HOBN) peaks at 0.78 at a pH of 8.79, whereas at 10 °C it peaks at 0.80 at a pH of 9.00.

tion of the 3 molecular forms depends on the pH (Figure 6). The fractional contribution of the 3 molecular forms is different at different temperatures because the pK_as vary with temperature. At 35 °C, the fractional contribution of the uncharged form (HOBN) peaks at 0.78 at a pH of 8.79, whereas at 10 °C it peaks at 0.80 at a pH of 9.00. Therefore the relative contributions of the 3 molecular forms of morphine will be different in a fish or a frog at 10 °C relative to a dog at 37 °C (Figures 6 B and 7 B). More importantly, the efficacy of narcotics and their agonists and antagonists depends in part on the relative amount that is in the uncharged or free-base form (HOBN) because it is more lipid-soluble,[10] and this amount will differ at different temperatures.

Effect of Temperature on Blood pH

The situation is made even more complex by the fact that the pH of blood of ectotherms is not constant but rather increases as temperature decreases in a fashion approximately parallel to the dissociation constant of water—the so-called alphastat effect.[11,13] The fish or frog at 15 °C has a blood pH of about 7.7 whereas the dog at 37 °C has a blood pH of 7.4. The fraction of morphine in the uncharged or free-base form (HOBN) is about 19% in the dog, whereas is it almost twice that (33%) in the fish or frog.

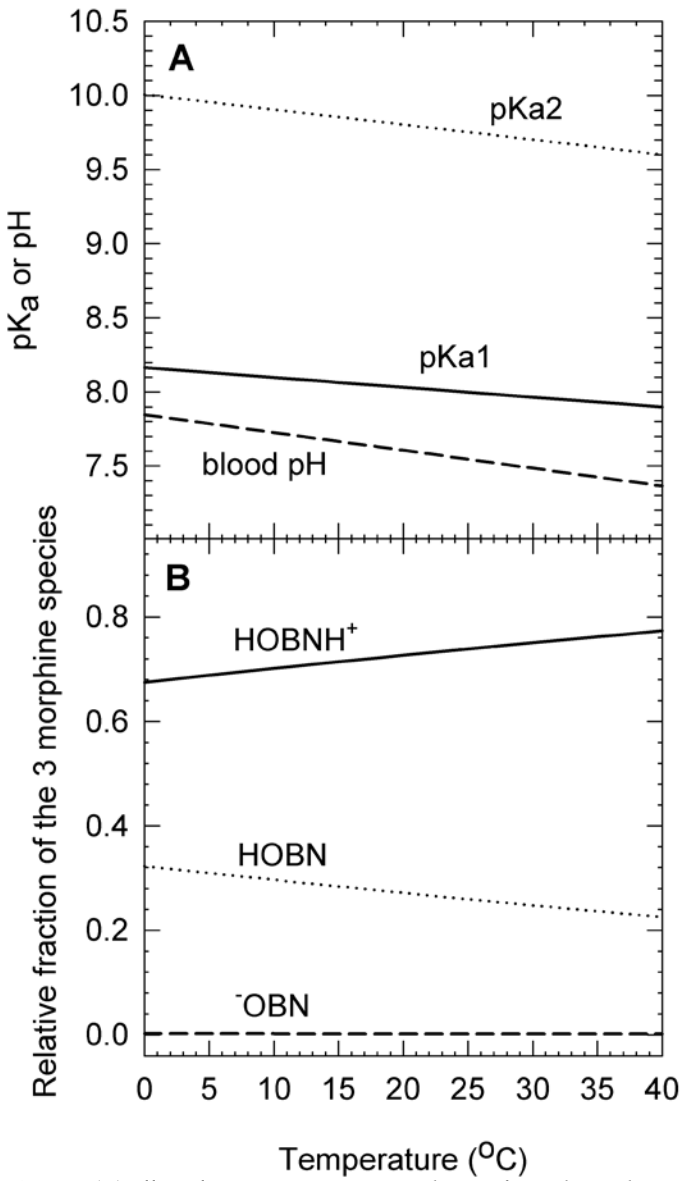

Figure 7. (A) Effect of temperature on pK_{a1} and pK_{a2} of morphine relative to its effect on blood pH in fish. The blood pH values for fish represent the average of published values, and the effect of temperature differs somewhat for different species. (B) Effect of temperature and blood pH on the relative contribution of the different species of morphine.

The effect of temperature on pK_{a1}, pK_{a2}, and blood pH is illustrated in Figure 7. At all temperatures, the contribution of $^{-}$OBN is negligible at physiologic pH values in mammals and ectotherms. However, the fractional contribution of $HOBNH^{+}$ increases markedly with temperature, and this change is associated with a concomitant fall in the fractional contribution of uncharged or free-base form (Figure 7). Therefore, the fraction of morphine in the free-base form is about twice as high in a fish or frog at 5 °C compared with a mammal at 37 °C—this difference is likely of biological importance, because it will affect bioavailability to tissues.

In essentially all ectotherms that have been studied, the effect of temperature on blood pH shows an increase in blood pH with a decrease in ambient (and thus body) temperature. The slope of this relation dpH/dT is different in different classes of vertebrates

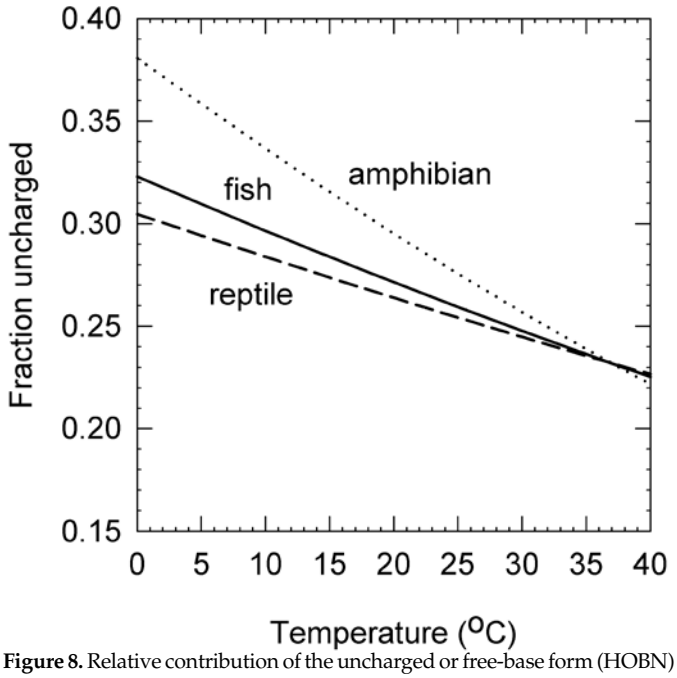

Figure 8. Relative contribution of the uncharged or free-base form (HOBN) of morphine decreases with an increase in temperature in all ectothermic (that is, cold-blooded) vertebrates. The values shown here are averages for the different classes; there is considerable variation between species within each class.

and ranges from –0.007 to –0.014 $°C^{-1}$ in fish to –0.011 to –0.018 $°C^{-1}$ in amphibians and –0.005 to –0.018 $°C^{-1}$ in reptiles.[3] The effect of temperature on uncharged or free-base form is shown in Figure 8 by using the mean slope for the data for fish, amphibians, and reptiles. These calculations illustrate the point that it is important to take into consideration the animal species, the ambient and body temperatures, and the blood pH of the animal in question.

Conclusions

Morphine, and many other pharmacologic agents, exists in different forms or species, and the relative amounts of the different forms depend on pH and temperature. Some forms are more efficacious because they are uncharged and can penetrate lipid membranes more easily than charged forms. Persons who administer pharmacologic agents to ectotherms (that is, cold-blooded animals) should consider the effect of temperature on the relative amounts of the different forms. Moreover, because the pH of blood, plasma, and tissues in ectotherms increases with a decrease in temperature, the combined effect of temperature and pH on speciation also is important. Finally, much remains to be learned about opioids in lower vertebrates.

Acknowledgments

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