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Embryo–Maternal Distribution of Basic Compounds in the CD-1 Mouse: Doxylamine and Nicotine

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Embryo–Maternal Distribution of Basic Compounds in the CD-1 Mouse: Doxylamine and Nicotine. ROBERTS, L. G., LUCK, W., HOLDER, C. L., SCOTT, W. J., NAU, H., AND SLIKKER, W., JR. (1989). *Toxicol. Appl. Pharmacol.* **97**, 134–140. The intracellular pH of the early postimplantation rodent embryo (pH_i) is alkaline with respect to the corresponding plasma of the pregnant dam. This transplacental pH gradient is of considerable importance in the accumulation of teratogenic weak acids by the embryo. The importance of pH in the partitioning of basic drugs across the early mammalian placenta has not been investigated. Theoretically, the maternal plasma should retain a higher concentration of basic drugs than the embryo due to a greater degree of drug ionization in the more acidic plasma. To explore the significance of pH partitioning upon the transplacental distribution of basic compounds, two bases, doxylamine and nicotine, were administered to pregnant CD-1 mice during early organogenesis. The maternal plasma and embryonic concentrations of the bases were measured and the resulting embryo/maternal plasma (E/P) ratio was calculated and compared to the ratio predicted by the Henderson–Hasselbalch equation. Following ip injection of nicotine on Day 9 of gestation, the E/P ratio was significantly greater than the predicted ratio 10 min after injection and continued to rise for 3 hr. For doxylamine succinate administered by oral gavage on Day 9 or 10, the E/P ratio was also significantly greater than the ratio predicted from the pH gradient. Our results indicate that the partitioning of these basic compounds between the maternal plasma and the early postimplantation rodent embryo is not a consequence of the pH gradient between the two compartments alone.

Embryonic concentrations of teratogens are of current interest in the field of teratology. Embryonic drug concentration has been implicated as a factor in determining the embryotoxicity of retinoic acid isomers (Creech-Kraft *et al.*, 1987), valproic acid (Nau, 1986), salicylic acid (Kimmel and Young, 1983; Wilson *et al.*, 1977), and glucocorticoids (Rowland *et al.*, 1983). Physiochemical factors which may interact to influence the transplacental distribution of drugs and toxic agents are the degree of ionization of the compound and the pH of the various body compartments. Since the nonionized form of a drug tends to penetrate a membrane barrier such as the placenta more quickly than the ionized form, a concentration gradient is largely dependent upon the difference in concentrations of the ionized form in each compartment (Mirken and Singh, 1976). The degree of ionization, in turn, is dependent upon the pH maintained within each compartment.

The intracellular pH of the early postimplantation rodent embryo (pH_i) is alkaline with respect to the corresponding plasma of

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the pregnant dam (Scott *et al.*, 1987). This has been shown to be of considerable importance in the transplacental distribution of acidic drugs (Nau and Scott, 1986). In whole embryo cultures, manipulation of the pH of the culture medium, thereby changing the *in vitro* pH gradient between the medium and the cultured embryos, has also been shown to change embryonic drug concentration in a manner predicted by the Henderson–Hasselbalch equation (Brown, 1987).

The importance of pH in the partitioning of basic drugs across the early mammalian placenta has not been investigated. The exposure of the embryo and fetus to certain basic compounds may be detrimental to normal development, although no well-documented human teratogen is a weak base. Doxylamine succinate was the antihistaminic component in Bendectin, which was widely prescribed for the relief of morning sickness until litigation prompted its discontinuance in 1983 (Holmes, 1983). Prenatal exposure to nicotine via maternal smoking has been shown to be detrimental to the human fetus, lowering birth weight, decreasing postnatal growth, and increasing the incidence of spontaneous abortions and stillbirths (Martin, 1982).

The purpose of this study was to assess the importance of pH upon the partitioning of basic drugs between the maternal and the embryonic compartments by investigating the transplacental distribution in the mouse of two basic compounds, doxylamine succinate and nicotine.

METHODS

Timed pregnant CD-1 mice, purchased from Charles River (Wilmington, MA), were housed in light (12 hr light, 9:30 AM to 9:30 PM), temperature (72°F ± 2), and humidity (50% ± 5) controlled quarters. Food (Purina Mouse Chow) and water were provided *ad libitum*. The morning after overnight mating was considered to be Day 0 of gestation. The number of gravid mice per time point is indicated in Figs. 1A and 2A.

[^{14}C]Doxylamine succinate, sp act 32.47 mCi/mmol, was synthesized by Southwest Foundation for Research and Education (San Antonio, TX; purity 98.2%). Nonlabeled doxylamine succinate (Richardson and Merrell, Inc., Hatboro, PA; 99+% pure) was added to the radiolabeled drug for a total dose of 133 mg salt/kg body wt (382 μCi/kg). Mice were dosed by oral intubation on Day 9 or Day 10 of gestation and anesthetized with sodium pentobarbital at 1, 3, or 6 hr after dosing. Embryos were removed from the uterus, and a maternal blood sample was obtained from the abdominal aorta. A sample of maternal muscle tissue, removed from the thigh, was taken for an estimate of maternal tissue concentration. Maternal plasma, embryo samples, and muscle were stored at −20°C until analysis. The concentration of doxylamine in maternal plasma, pooled litter embryo homogenate, and muscle was determined by combined HPLC/liquid scintillation spectroscopy of the parent compound fraction as described by Holder *et al.* (1987).

Nicotine (Sigma Chemical Co., St. Louis, MO) was injected ip at a dose of 2.5 mg/kg on Day 9 of gestation. Mice were terminated at 10 min, 30 min, 1 hr, or 3 hr after injection. Maternal plasma and embryonic samples were collected and stored as described previously for doxylamine. The concentration of nicotine in these samples was determined by gas chromatography using a nitrogen-selective detector (Luck and Nau, 1984).

Mean embryo and maternal plasma drug concentrations were generated for each time point of sampling. The embryo/plasma concentration ratio (E/P) was calculated for each pair of samples collected from one female mouse. The mean E/P ratio over all sampling times was calculated when there was no statistical difference in the mean ratio values calculated for a drug at different sampling times, and if the data did not suggest a chronological trend for the E/P ratio that would countersuggest equilibrium. The larger sample size of this cumulative mean resulted in a smaller confidence interval and allowed a more rigorous test of the pH partitioning hypothesis. The predicted E/P ratio was calculated from the Henderson–Hasselbalch equation using the form

$$\text{E/P ratio} = \frac{1 + 10(pK_a - pH_i)}{1 + 10(pK_a - pH_{plasma})}$$

using the pH of mouse plasma and pH_i of embryos as reported by Nau and Scott (1987). The change in the predicted ratio reflects the changes, from Day 9 to 10, of plasma pH (7.27 to 7.33) and embryonic pH_i (7.65 to 7.46). Embryo pH_i was determined by the method of weak acid (5,5-dimethyl-2,4-oxazolidinedione, DMO) distribution, an indirect method that yields an average pH_i for the whole embryo and which has shown agreement with values determined by microelectrode techniques for various tissues (Roos and Boron, 1981). The pH partitioning hypothesis was rejected for a basic compound if the predicted E/P ratio did not fall within the 95% confidence limits of the actual E/P mean ratio. The degree of statistical significance was determined by the *t* test, using the formula (Snedecor and Cochran, 1967)

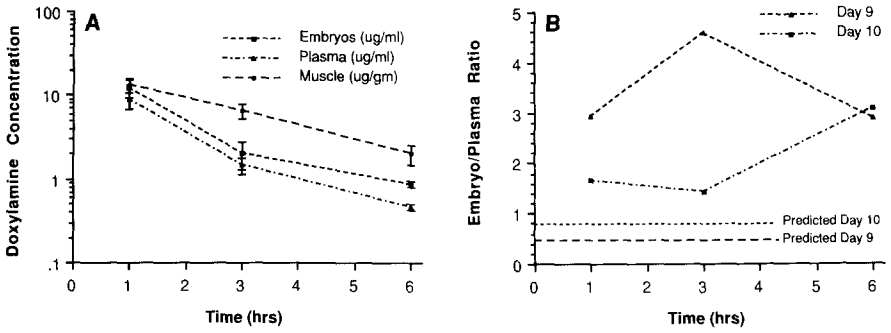

FIG. 1. (A) The concentration of doxylamine in maternal plasma or pooled litter embryo homogenate collected after po administration of doxylamine succinate at 133 mg/kg on Day 10 of gestation. The mean ± standard error for each sample is shown on the graph. $n = 4$ at 1, 3, and 6 hr. (B) The embryo/plasma (E/P) ratios for doxylamine measured at 1, 3, and 6 hr after treatment with doxylamine succinate on Day 9 or 10 of gestation. As there was no significant difference between the ratios that were calculated at each sampling time, the 95% confidence limits, 2.01 to 4.53 on Day 9, and 1.25 to 2.86 on Day 10, were determined from the data for all three time points on each day ($n = 9$, Day 9; $n = 12$, Day 10). The predicted E/P ratios, 0.42 (Day 9) and 0.74 (Day 10), were calculated as described under Methods.

$$t = \frac{\text{Actual E/P} - \text{Predicted E/P}}{\text{Standard error of the mean}}$$

$$\text{degrees of freedom} = n - 1.$$

The muscle/plasma ratio (M/P) was calculated in a similar manner. The predicted M/P ratio was calculated as described for embryo tissue, with the reported mouse muscle intracellular pH of 7.07 (Aickin and Thomas, 1977) used as the estimate of muscle pH_i.

RESULTS

Doxylamine succinate. The total concentrations of doxylamine as the free (no succinate) base in plasma, embryos, and muscle on Day 10 of gestation are shown in Fig. 1A. The concentration of the drug on Day 9 was approximately threefold greater in embryonic tissue than in maternal plasma (Fig. 1B). The E/P ratios calculated for each sampling time were not significantly different, suggesting that doxylamine had reached an equilibrium between the embryonic and the maternal compartments by 1 hr after treatment. The E/P ratio predicted from the Henderson–Hasselbalch equation was less than the lower limit for the 95% confidence interval ($p < 0.0005$; Table 1). Similar results were found for the doxylamine E/P ratio following treatment on Day 10 (Fig. 1B). There was no statistically significant difference between the E/P ratios calculated at 1, 3, and 6 hr after treatment. As for Day 9, the E/P ratio predicted from the Henderson–Hasselbalch equation was less than the lower limit for the 95% confidence interval, although the difference between the predicted and the measured ratios on Day 10 was less than that for Day 9 ($p < 0.005$). The predicted ratios on Day 9 and Day 10 differ because of the change that occurs in plasma pH and embryonic pH_i during this time.

The concentration of doxylamine in muscle was greater than in either embryos or plasma and declined less rapidly. The M/P ratio increased from 1.65 ± 0.545 at 1 hr to 5.88 ± 4.25 at 3 hr and to 6.75 ± 3.95 at 6 hr after dosing. Only the M/P ratio at 1 hr was similar to the predicted ratio of 1.81, although only the 6 hr M/P ratio was significantly different from the predicted ratio.

Nicotine. The total nicotine concentration in maternal plasma initially declined rapidly after injection, but disappeared slowly between 30 min and 3 hr (Fig. 2A). The embry-

TABLE 1

THE PREDICTED AND ACTUAL RATIOS OF DOXYLAMINE AND NICOTINE BETWEEN EMBRYONIC TISSUE AND MATERNAL PLASMA (E/P)

Chemical	pK_a	Predicted E/P Day 9	Actual E/P Day 9	Predicted E/P Day 10	Actual E/P Day 10
Doxylamine	9.2[a]	0.42	3.27[b,c] (2.01–4.53)[e]	0.74	2.06[b,d] (1.25–2.86)
Nicotine	7.84[a]	0.54	5.40[f,g] (4.24–6.56)	0.80	—

[a] Only the pK_{a_1} is reported.
[b] The mean value was determined from samples collected at all three sampling times.
[c] $p < 0.0005$.
[d] $p < 0.005$.
[e] Numbers in parentheses are the upper and lower 95% confidence limits for the mean E/P ratio of the sample.
[f] The lowest ratio measured for nicotine, 10 min after injection.
[g] $p < 0.001$.

onic concentration of nicotine displayed a similar initial decline, but remained steady from 1 to 3 hr.

Much higher concentrations of nicotine were measured in embryonic tissue than in maternal plasma (Fig. 2B). The measured E/P ratio increased throughout the postinjection interval. Each mean ratio calculated for each sampling time was significantly different from the predicted ratio (Table 1); the mean ratios for each time point were compared to the predicted ratio individually because of the large differences in the measured values and the trend for the change over time.

DISCUSSION

It is apparent that the pH partitioning hypothesis does not describe the transplacental distribution of these basic compounds (Table 1). Neither of the bases, of different pK_a's,

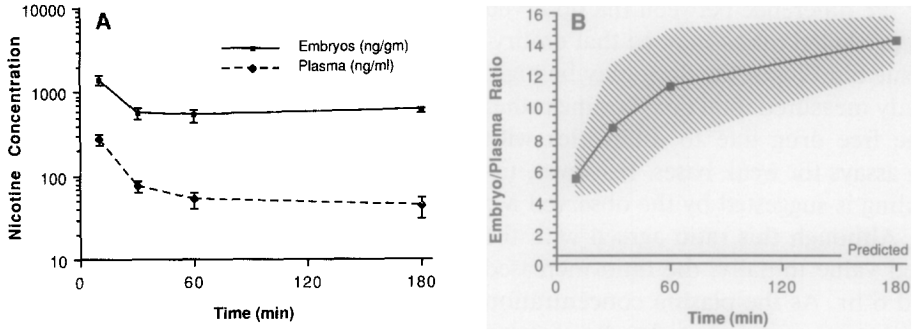

FIG. 2. (A) Nicotine concentrations in embryo homogenates or maternal plasma following ip administration of nicotine, 2.5 mg/kg, on Day 9 of gestation. The mean ± standard error for each sample is shown on the graph. The sample sizes were $n = 5$ (10 min), $n = 6$ (30 min), $n = 7$ (1 hr), and $n = 4$ (3 hr). (B) The E/P ratios for nicotine distribution following ip injection at 2.5 mg/kg on Day 9 of gestation. The mean ± 95% confidence intervals (hatching) were calculated separately for each time point. The predicted E/P ratio, 0.54, was calculated as described under Methods. The sample sizes were those of Fig. 2A.

partitioned between the embryonic and the central maternal compartments in a manner predicted from the difference between plasma pH and embryonic pH_i. This is unlike the situation for weak acids, for which pH partitioning does predict the concentration gradients between embryonic tissue and maternal plasma (Nau and Scott, 1986).

The transplacental partitioning of doxylamine has been investigated previously in the late gestational rhesus monkey (Slikker *et al.*, 1986). At this stage of gestation, fetal plasma is more acidic than maternal plasma by approximately 0.1 to 0.15 pH units. Theoretically, this should lead to a greater concentration of the base on the fetal side of the placenta. In reality, however, the reverse was observed, although the data suggested that equilibrium had been achieved. Thus it would appear that the pH gradient maintained across the placenta does not control the distribution of doxylamine to the rhesus fetus.

In this study, the more alkaline character of the mouse embryonic pH_i was predicted to prevent the accumulation of doxylamine in the embryonic compartment. This was not the case. A greater concentration of doxylamine was found in embryonic tissue than in plasma and the E/P ratio did not change over time, suggesting that the two compartments were in equilibrium with each other. One caveat for the difference between the observed and the predicted ratios may be that embryonic tissue binding of the drug may be high. This study measured total doxylamine rather than the free drug due to difficulties with binding assays for weak bases. However, tissue binding is suggested by the observed M/P ratio. Although this ratio agreed with the predicted value initially, the ratio increased at 3 and 6 hr. As the plasma concentration decreased over time, muscle doxylamine concentration decreased at a slower rate; one explanation for this could be tissue binding of doxylamine in muscle.

Transplacental concentrations of nicotine have been explored under a variety of experimental situations, primarily because of the association between maternal smoking and adverse fetal effect. It has been measured in higher concentrations in amniotic fluid, placental tissue, and umbilical vein serum than in maternal serum during both the second trimester of pregnancy and at birth (Luck and Nau, 1984). This agrees with the idea of "ion trapping" in these compartments when they are more acidic than maternal plasma. In the rabbit, [^{3}H]nicotine has been found to accumulate in the uterine fluid on Day 6 (preimplantation) of pregnancy (McLachlan *et al.*, 1976). This was suggested to be due to the binding of nicotine to pregnancy-specific uterine fluid proteins, as no accumulation occurred in the uterine fluid of nonpregnant does. The theoretical uterine fluid/plasma ratio would be less than one due to the slightly more alkaline character of the uterine fluid; therefore, pH partitioning does not explain the actual ratio. However, this same group (Fabro and Sieber, 1969) was able to show that nicotine is largely excluded from within the rabbit blastocyst and attributed the difference in concentration between the uterine fluid and the blastocysts of pregnant rabbits to an ion-trapping mechanism. In the late gestational (Days 14–18) mouse, nicotine has been found to accumulate in the placenta and to a lesser extent in the fetus relative to the concentration detected in maternal plasma (Tjalve *et al.*, 1968). Fetal accumulation occurred primarily in the lung, trachea, larynx, and intestine. Nicotine has been reported to be highly bound in lung tissue (Hori *et al.*, 1987). The E/P ratios measured during this period were similar to our ratio measured on Day 9. This suggests that the period of pregnancy does not influence the transplacental distribution of nicotine, as well as indicating that the pH gradient, which changes during gestation, does not alter the E/P ratio for nicotine. The conclusion of Weathersbee and Lodge (1979), that only negligible amounts of nicotine cross the placenta, would appear to be unfounded.

One reason for the deviation of these bases from the partitioning predicted solely from pH may be that the concentrations reported herein are for total drug rather than the unbound portion. The plasma protein binding of nicotine is low (Schievelbein, 1984; McLachlan *et al.*, 1976; Short and Tumbleson, 1973), while the plasma protein binding of doxylamine is likely to be high (Paton and Webster, 1985). Any plasma binding would decrease the unbound plasma concentration and hence increase the deviation of the ratio from the predicted value. Tissue binding within the embryo is much more likely to be a factor. Many basic drugs accumulate in tissue; one specific intracellular binding site is the mitochondria (Hori *et al.*, 1987), which will accumulate lipophilic, basic drugs. Tissue binding is also supported by the accumulation of [^{3}H]nicotine in the tissue fraction of the rabbit blastocyst (McLachlan *et al.*, 1976) while the fluid fraction concentration was comparatively low. Nearly all nicotine was found to be bound to blastocyst tissue; the concentration in the blastocyst fluid was approximately one-tenth of the tissue concentration. If the unbound fraction of nicotine in the rabbit blastocyst is used as an estimate of the unbound fraction of nicotine in the mouse embryo, then the estimated unbound nicotine E/P ratio at 10 min after injection would be 0.54, the same as the predicted ratio. It may be that the unbound fractions of these bases within the embryo were small. Total drug concentration, while demonstrating that the pH gradient between the maternal plasma and the embryo does not prevent drug accumulation by the embryo, may not accurately depict the equilibrium between the plasma and the embryo that may be reached by the unbound form.

In summary, the partitioning of basic compounds between the maternal plasma and the developing embryo does not appear to be a consequence of the pH gradient. Unlike weak acids, for which the transplacental distribution of the free fraction is predictable from the Henderson–Hasselbalch equation, other factors, most likely the binding of basic compounds intracellularly, play a more major role in the partitioning of bases between the embryonic and the maternal compartments.

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