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SHORT COMMUNICATIONS

Relation between molecular weight and pulmonary absorption rate of lipid-insoluble compounds in neonatal and adult rats

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A previous investigation from this laboratory revealed a marked difference between newborn and more mature rats with regard to the permeability of the respiratory tract to certain nonvolatile drugs [1]. It was shown that lipid-insoluble compounds such as tetraethylammonium, mannitol and *p*-aminohippuric acid were absorbed approximately two times more rapidly in 3- to 12-day-old rats than in animals 18 days of age or older. In contrast, lipid-soluble drugs such as procaine amide and sulfisoxazole were absorbed at similar rates in newborn and adult rats. Since the lipid-insoluble compounds appear to be absorbed by diffusion through aqueous membrane channels (pores) rather than through lipoid regions of the absorbing membrane [2], it was concluded that the respiratory tract epithelium of the younger rats has a greater porosity than that of older animals. The present study, which extends the previous work to include a variety of lipid-insoluble compounds of widely different molecular weights, shows that the age-related difference in permeability applies only to drugs in a certain molecular weight range. The results provide new information about the porous nature of the respiratory tract epithelium.

Pulmonary absorption of radioactively labeled compounds was measured in 6-day-old (10–12 g) and adult (150–200 g) male Sprague–Dawley rats using methods described in detail in previous publications from this laboratory [1, 3]. In brief animals were anesthetized with pentobarbital, and the trachea was exposed through an incision along the ventral aspect of the neck. A tight-fitting tracheal cannula made from polyethylene tubing was inserted to a depth of one-fourth the cannula length through an incision between the fourth and fifth tracheal rings caudal to the thyroid cartilage. Body temperature was maintained at 37 ± 1° by heat from an incandescent lamp in a reflector suspended over the animal. For administration of a compound into the lungs, a radioactively labeled compound together with the unlabeled compound was dissolved (total concentration 0.1 to 1 mM) in a modified Krebs–Ringer phosphate solution, pH 7.4. With the animal resting on its back, the solution at 37° was injected into the lungs through a length of fine polyethylene tubing attached to the needle of a calibrated microliter syringe. For an injection, the tubing was inserted through the tracheal cannula to a point 1–2 mm above the bifurcation of the trachea, and the solution was injected over a 1- to 2-sec interval. The tubing was then completely withdrawn, and the animal was maintained under light anesthesia for the remainder of the experimental period, which lasted as short a time as 1 min for a rapidly absorbed drug and as long as 10 hr for a slowly absorbed compound. The volume of solution injected (10 μl in neonatal rats and 100 μl in adults) was approximately 1% of lung volume [4]. The validity of the intratracheal injection technique for comparative studies in rats of various ages has been established previously [1].

At the end of an absorption period, the blood supply to the lungs was severed quickly. The lungs and attached trachea were excised and homogenized together with sufficient distilled water to make a total weight of 4 g in adults and 2.5 g in newborns. Samples of the tissue homogenate were digested with perchloric acid and hydrogen peroxide and mixed with a liquid scintillation medium, and the resulting solutions were assayed for radioactivity using a Packard model C2425 Tri-carb liquid scintillation spectrometer as described previously [1]. When known amounts of labeled compounds were added to neonatal or adult lung–trachea preparations and the tissues assayed as described above, recoveries were complete (97–100%).

Each drug was studied in twelve to fifteen 6-day-old or twelve to eighteen adult rats. Semilogarithmic plots of percentage of dose unabsorbed against time [3] resulted in straight lines for all compounds in both neonatal and adult animals. Straight-line plots have been reported previously (adult rats only) [3, 5] for all the compounds except guanidine, which was not included in prior studies of pulmonary absorption. Half-times for absorption and apparent first-order absorption rate constants, k, were calculated from the slopes of the lines [3]. Statistical evaluations were made with Student's t-test [6].

[^{14}C]Guanidine HCl (sp.act. 48 mCi/mmole), [U-^{14}C]-erythritol (sp. act. 2.3 mCi/mmole), and cyano[G-^{3}H] cobalamin (sp. act. 4500 mCi/mmole) were obtained from the Amersham Corp., Arlington Heights, IL. [^{14}C]Urea (sp. act. 8 mCi/mmole), [U-^{14}C]sucrose (sp. act. 4.9 mCi/mmole), [*carboxyl*-^{14}C]inulin (sp. act. 2.5 mCi/g, approximate mol. wt 5,250), and [*carboxyl*-^{14}C]dextran (sp. act. 2.16 mCi/g, approximate mol. wt 20,000) were obtained from the New England Nuclear Corp., Boston, MA. Unlabeled compounds were obtained from the Sigma Chemical Co., St. Louis, MO, except that no unlabeled material was used with the labeled inulin or dextran.

Absorption rates of lipid-insoluble compounds in 6-day-old and adult rats are summarized in Table 1, in which the compounds have been listed in increasing order of molecular weight. Included in the table are results for three additional compounds, tetraethylammonium, mannitol and *p*-aminohippuric acid, which were investigated previously [1] using the same experimental method as in the present study. Relative absorption rates covered a wide range of values. For example, the most rapid rates (k = 6.6–10.1), obtained with guanidine and urea, were more than 100 times faster than the slowest rates (k = 0.0604–0.0655), obtained with dextran. Inspection of the data shows that over the entire range of molecular weights studied, absorption rates of compounds tended, for the most part, to rank in the inverse order of their molecular weights for both neonatal and adult animals.

When absorption rates (k) in newborn rats are compared with those obtained in adult animals (Table 1, last column), the compounds can be divided into three distinct groups according to molecular weight: (1) the smallest molecules (mol. wt 59–60), which were absorbed at about the same rates in neonates and adults (ratio of k values = 1.1–1.2); (2) compounds with molecular weights in the range of 122–1,355, which were absorbed approximately two times faster in newborns than in adults; and (3) the largest molecules (mol. wt 5,250–20,000), which were absorbed at

Table 1. Relation between molecular weight and pulmonary absorption rate of compounds in neonatal and adult rats

Compound	Molecular weight	Rate of absorption* Neonatal Half-time (min)	Neonatal $k \times hr^{-1}$	Adult Half-time (min)	Adult $k \times hr^{-1}$	Ratio, $\frac{k_{neonatal}}{k_{adult}}$
Guanidine	59	5.1 ± 0.2	8.15	6.3 ± 0.2	6.60	1.2
Urea	60	4.1 ± 0.2	10.1	4.7 ± 0.3	8.85	1.1
Erythritol	122	22 ± 2	1.89	37 ± 3	1.12	1.7†
Tetraethylammonium‡	130	28 ± 2	1.49	63 ± 4	0.660	2.3†
Mannitol‡	182	32 ± 2	1.30	60 ± 6	0.693	1.9†
p-Aminohippuric acid‡	194	22 ± 2	1.89	41 ± 4	1.01	1.9†
Sucrose	342	37 ± 2	1.12	84 ± 7	0.495	2.3†
Cyanocobalamin	1355	101 ± 8.3	0.412	190 ± 18	0.219	1.9†
Inulin	5250	211 ± 12	0.197	220 ± 13	0.189	1.0
Dextran	20000	635 ± 53	0.0655	688 ± 51	0.0604	1.1

* Rates are expressed both as a half-time ± S.E.M. in twelve to eighteen animals and as a first-order absorption rate constant, k, which is equal to 0.693 divided by the half-time in hours.

† Ratio is significantly greater than unity ($P < 0.05$).

‡ Data are taken from a previous publication from this laboratory [1].

about the same rates in the two age groups.

The compounds in Table 1 have such extremely low organic solvent-to-water partition coefficients [7–9] that they are considered to be essentially lipid insoluble and thus able to diffuse across biologic membranes only by way of aqueous membrane channels (pores) [10]. In the respiratory tract, the pores of the epithelial membrane appear to be of more than one size. For example, a study of the permeability of the saline-filled adult dog lung to urea and three saccharides [11] has suggested a minimum of two pore populations consisting of many small pores and relatively few large pores. Moreover, previous work in this laboratory with the normal air-filled adult rat lung [3] has suggested that at least three populations of pore size are needed to explain absorption data obtained with several lipid-insoluble compounds. Results of the present study suggest the presence of three populations of pore size with the following relative numerical distribution: (1) numerous small pores, those that admit urea and guanidine but exclude compounds of mol. wt 122 or above; (2) a few medium-size pores, those that admit compounds with mol. wt as high as 1,355 but exclude compounds of mol. wt 5,250 or higher; and (3) very few large pores, those that admit inulin and dextran (mol. wt 5,250–20,000) as well as smaller molecules. With this model, only the medium-size pores would be more numerous (or larger) in neonates than in adults. For example, with the medium-size pores being twice as numerous (or having twice the cross-sectional area) in newborns as in adults, absorption of drugs in the mol. wt range 122–1,355 would be twice as fast in the young animals as in the older ones, whereas the absorption rates of inulin and dextran would not be different in the two age groups, because these substances do not have access to the medium-size pores. Furthermore, the absorption rates of small molecules like urea and guanidine would not differ appreciably in the two age groups, because nearly all the absorption would be accounted for by the numerous small pores. Although other, more complicated pore distribution models might be consistent with the present results, the above idea provides a relatively simple working hypothesis to be evaluated in future investigations.

Regardless of the precise nature of the pores in the respiratory tract epithelium, it is clear that the newborn rat lung is approximately twice as permeable as the adult lung to most lipid-insoluble drugs, those in the mol. wt range of 122–1,355. As shown previously [1], this permeability difference disappears rather abruptly at an age of approximately 15 days when the lung takes on adult permeability characteristics. Additional studies have shown that thyroxine or cortisone treatment of newborn rats accelerates the development of adult permeability characteristics so that the exaggerated absorption rates for lipid-insoluble drugs disappear at age 3–9 days instead of at age 15 days [12, 13].

The physiological significance of a relatively high permeability to lipid-insoluble substances in the newborn rat lung is not yet apparent. Perhaps it is important for reabsorption of certain biochemical substances that reach the inner surface of the lung by secretory processes or other pathways. Whatever the functional significance, if this high permeability of the neonatal lung extends to newborn humans, there would be a number of important implications regarding the systemic effects of inhaled therapeutic agents and toxic materials.

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Phosphorylation of "tricyclic nucleoside" by adenosine kinases from L1210 cells and HEp-2 cells

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"Tricyclic nucleoside" (TCN) [6-amino-4-methyl-8-(β-D-ribofuranosyl)-(4*H*, 8*H*)pyrrolo[4, 3, 2-*de*]pyrimido [4, 5-*c*]pyridazine] is a nucleoside of novel structure synthesized by Schram and Townsend [1]. This compound is active against experimental animal tumours [2] and is currently in Phase I clinical trial. The active form of TCN is its 5'-phosphate, the formation of which is catalyzed by adenosine kinase [3, 4]. Several years ago we reported an apparent Michaelis constant of 215 μM for the phosphorylation of TCN by a partially purified adenosine kinase from HEp-2 cells [4]. More recently we have had occasion to study the phosphorylation of TCN by kinase preparations from both HEp-2 cells and L1210 cells and have found Michaelis constants for both enzymes two orders of magnitude less than that previously reported for the enzyme from HEp-2 cells.

For this study we used an adenosine kinase preparation purified 134-fold from HEp-2 cells [5] and a preparation purified to apparent homogeneity from L1210 cells [6]. The assays measured the conversion of [^{14}C-methyl]TCN (obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) to its 5'-phosphate. The conditions of the assays, which have been reported earlier [5–7], differed for the enzymes from the two sources. For the enzyme from HEp-2 cells [5] the assays were performed at 25°; the incubation mixture contained 2.5 mM ATP, 0.25 mM $MgCl_2$, and 50 mM potassium phosphate buffer; and the nucleotide formed was isolated by paper chromatography and assayed for radioactivity in a chromatogram scanner. For the enzyme from L1210 cells [6, 7], the assays were performed at 37°; the incubation mixture contained 1.25 mM MgATP, $MgCl_2$ in 0.4 mM excess of that of MgATP, 50 mM Tris–HCl, pH 8.0, and bovine serum albumin (6 μg); and the nucleotide formed was isolated on Whatman DE-81 paper discs which were assayed for radioactivity by liquid scintillation spectrometry. The conditions for assay of the HEp-2 enzyme were not changed to those in current use because we were attempting to determine the reasons for the discrepancy between our present and earlier results.

Double-reciprocal plots for the phosphorylation of TCN by the two enzyme preparations are shown in Fig. 1. For the HEp-2 enzyme the apparent K_m for TCN was 1.8 μM and the V_{max} was 42% that for adenosine; the apparent K_m for adenosine (plot not shown) was 1.7 μM. For the L1210 enzyme the apparent K_m for TCN was 1.3 μM and the V_{max} was 110% that of adenosine; the apparent K_m for adenosine (plot not shown) was 0.5 μM [7].

We have examined our earlier data with the HEp-2 enzyme and have found no apparent error, and we are therefore unable to explain the discrepancy between our earlier and present results. In our former study, we used a different assay for product formation (determination of formation of [^{32}P]TCN 5'-phosphate when the incubation mixture contained [γ-^{32}P]ATP and unlabeled TCN), but this method of determining kinase activity is unlikely to be

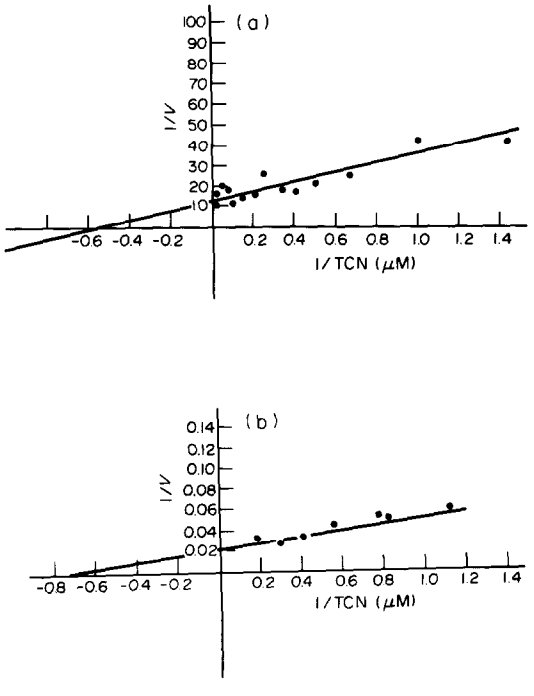

Fig. 1 Double-reciprocal plots of initial TCN concentration vs the reaction velocity (nmoles TCN-phosphate per min per mg protein) for the phosphorylation of TCN by adenosine kinase preparations from HEp-2 cells (A) and L1210 cells (B). See text for the conditions for the assays and the kinetic constants calculated from these plots.