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Postnatal Development of Carrier-Mediated Absorption of Disodium Cromoglycate from the Rat Lung (40537)[1]

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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). For example, animals 12 days of age or younger were shown to absorb a number of lipid-insoluble compounds approximately two times more rapidly than rats 18 days of age or older.

Since the previous study dealt only with substances that cross the pulmonary membrane by a process of diffusion, it did not approach the question of developmental changes that might occur in more complex absorption processes such as carrier-mediated transport. Studies with other organs, such as the liver (2) and kidney (3), have revealed quantitative differences between newborn and older mammals with regard to the ability to transport compounds by carrier-mediated processes. Accordingly, the present investigation was undertaken to examine the influence of age on the pulmonary absorption of disodium cromoglycate (DSCG; cromolyn sodium), an antiasthmatic drug that is used clinically by inhalation and which has previously been shown to be absorbed from the lung of adult rats in part by a carrier-type transport process (4).

Materials and methods. Pulmonary absorption of disodium cromoglycate (DSCG) was measured in animals of various ages and of either sex using a method described in detail in a previous publication from this laboratory (1). In brief, Sprague–Dawley rats were anesthetized with sodium pentobarbital, and the trachea was exposed through a longitudinal 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 a transverse incision between the fourth and fifth tracheal rings caudal to the thyroid cartilage. For administration of a compound into the lungs, test solutions of DSCG (0.05–100 m*M*) were prepared by adding a tracer amount of the ^{3}H-labeled compound together with unlabeled compound to Krebs–Ringer phosphate solution (pH 7.4) in which Ca^{2+} and Mg^{2+} ions had been omitted to avoid formation of insoluble salts with DSCG (5). Drug solutions were injected into the lungs through a length of polyethylene tubing attached to a calibrated 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 injected over a 1- to 2-sec interval. The volume (μl) of solution injected intratracheally was varied in order to maintain a nearly constant relationship between injected volume and lung volume in rats of various ages. Thus, using values of lung volume reported by Burri *et al.* (6), the volume of solution injected was approximately 1% of lung volume in animals of various ages. The validity of the intratracheal injection technique in rats has been established previously (1). Body temperature was monitored continuously with a thermistor probe and telethermometer and was maintained at 37 ± 1° by heat from a 40 W incandescent lamp in a reflector suspended over the animal at a distance of about 25 cm (1).

At the end of an absorption period (0–60 min), the lungs and attached trachea were removed from the animal, homogenized, digested, and assayed for unabsorbed drug by liquid scintillation spectrometry using a method described previously (1). When known amounts of [^{3}H]DSCG were added to lung tissue and the assay carried out as described above, recoveries were complete (98–103%). Extensive chromatographic studies of

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the metabolic fate of [^{3}H]DSCG have indicated that the compound is metabolically stable in the rat (7, 8).

To examine the effect of various other anions on the absorption of DSCG, each unlabeled compound was dissolved in the buffer solution together with the [^{3}H]DSCG before intratracheal injection. Compounds were obtained from the following sources: phenol red sodium salt, J. T. Baker Chemical Co., Phillipsburg, New Jersey; benzylpenicillin potassium, Nutritional Biochemicals Corp., Cleveland, Ohio; *p*-aminohippuric acid, Eastman Organic Chemicals, Rochester, New York. Labeled disodium cromoglycate ([^{3}H]DSCG), sp act 43.5 mCi/mmole, and the unlabeled compound were kindly provided by Fisons Ltd., Loughborough, England.

Statistical evaluations were made using Student's *t* test.

Results. Semilogarithmic plots describing the pulmonary absorption of ^{3}H-labeled DSCG as a function of time in 6-day-old rats are shown in Fig. 1. Since the plotted data for the two concentrations studied (0.05 and 50 m*M*) conformed to straight lines, relative absorption rates could be calculated from the slope of the lines and expressed as half-times. At the lower concentration, the absorption half-time was 27 min; and at the higher concentration, the half-time was 56 min. The markedly slower rate of absorption seen with the higher concentration indicated that, as previously reported for adult rats (4), DSCG is absorbed, at least in part, by a process that can be saturated.

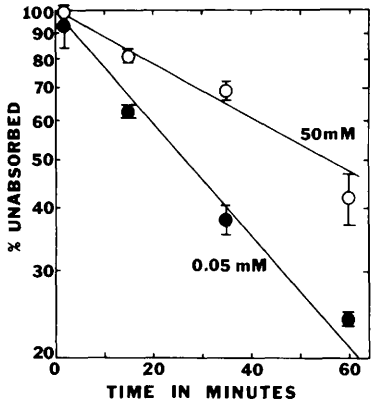

FIG. 1. Effect of concentration on rate of absorption of [^{3}H]DSCG from the lungs of 6-day-old rats. Modified Krebs–Ringer phosphate solution (0.01 ml), containing either 0.05 or 50 m*M* compound, was administered through a tight-fitting tracheal cannula to anesthetized rats. Each point is the mean ± SE for three to eight animals.

To assess the quantitative significance of the saturable absorption process in 6-day-old rats, the 35-min absorption of DSCG was measured for a number of concentrations ranging from 0.05 to 100 m*M*. As shown in Table I, as the concentration was increased from 0.05 to 25 m*M*, the percentage of the administered dose absorbed declined from 65.4 to 30.7. However, when the concentration was further increased, from 25 to 100 m*M*, the percentage absorption tended to remain constant at approximately 29% (range 26–30.7). This suggested that, at the higher concentrations, the saturable absorption process had become saturated, and that most of the drug was being absorbed by a process of simple diffusion.

To examine the specificity of the saturable transport process in 6-day-old animals, the 35-min absorption of DSCG was studied in the presence of various other organic anions (Table II). Phenol red and benzylpenicillin, in concentrations of 1 and 10 m*M*, inhibited the overall absorption of 0.05 m*M* DSCG by 13.6–22.6%, and they inhibited the saturable component of absorption by 24.5–40.7%. *p*-Aminohippuric acid did not significantly inhibit the absorption of 0.05 m*M* DSCG when present in a concentration of 10 m*M*; however, it did depress the saturable absorption process by 29.7% when the concentration was increased to 50 m*M*.

In Table III is shown the effect of age on the 35-min pulmonary absorption of 0.05 and 50 m*M* DSCG. Since, as shown above, the saturable absorption process is saturated at

TABLE I. EFFECT OF CONCENTRATION ON PULMONARY ABSORPTION OF DSCG IN 6-DAY-OLD RATS

Conc. of DSCG (m*M*)	No. of animals	Percentage of dose of DSCG absorbed in 35 min[a]
0.05	17	65.4 ± 1.3
0.5	4	51.6 ± 2.3
1.0	4	49.6 ± 5.6
25.0	6	30.7 ± 1.6
50.0	6	30.5 ± 3.6
100.0	5	26.0 ± 2.4

[a] Values represent mean ± SE.

TABLE II. EFFECT OF ANIONIC COMPOUNDS ON PULMONARY ABSORPTION OF DSCG IN 6-DAY-OLD RATS

Compound	No. of animals	Conc. of compound (m*M*)	Percentage of dose of 0.05 m*M* DSCG absorbed in 35 min[a]	Percentage inhibition of overall absorption of DSCG	Percentage inhibition of saturable component of DSCG absorption[b]
Control	17	—	65.4 ± 1.3		—
Phenol red	3	0.1	65.8 ± 4.2[c]	0	0
	7	1.0	51.3 ± 2.3	21.6	38.7
	4	10.0	50.6 ± 5.0	22.6	40.7
Benzylpenicillin	5	1.0	56.5 ± 4.1	13.6	24.5
	4	10.0	51.5 ± 3.6	21.3	38.2
p-Aminohippuric acid	6	10.0	62.9 ± 5.3[c]	3.8	6.9
	6	50.0	54.6 ± 4.9	16.5	29.7

[a] Values represent mean ± SE.
[b] Based on a diffusion component of absorption of 29% of the dose in 35 min (4).
[c] Not significantly different from the control value ($P > 0.05$).

TABLE III. EFFECT OF AGE ON PULMONARY ABSORPTION OF 0.05 AND 50 m*M* DSCG IN THE RAT

Age (days)	Percentage of dose of DSCG absorbed in 35 min: Overall absorption[a] 0.05 m*M* DSCG	Overall absorption[a] 50 m*M* DSCG	Absorption due to saturable component only (0.05 m*M* DSCG)[b]
1	62.6 ± 6.3 (3)	46.4 ± 5.8 (5)	16.2
3	55.2 ± 3.4 (10)	43.6 ± 3.4 (8)	11.6
6	65.4 ± 1.3 (17)	30.5 ± 3.6 (6)	34.9
12	74.6 ± 1.9 (6)	35.8 ± 4.8 (6)	38.8
18	57.9 ± 4.7 (6)	23.8 ± 3.0 (6)	34.1
27	65.0 ± 1.8 (5)	22.1 ± 2.2 (5)	42.9
Adult	61.4 ± 1.7 (3)	18.5 ± 2.5 (4)	42.9

[a] Values represent mean ± SE with the number of animals in parentheses.
[b] Overall absorption for 0.05 m*M* DSCG less that due to diffusion as given approximately by the overall absorption values for 50 m*M* DSCG (4).

concentrations above 25 m*M*, most of the DSCG absorption seen with the 50 m*M* solution is accounted for by simple diffusion (4). Results with 50 m*M* DSCG showed 30.5–46.4% absorption for rats 1–12 days of age and 18.5–23.8% absorption for rats 18 days old or older. This confirms previously reported results (1) that rats 12 days of age or younger absorb a number of lipid-insoluble compounds by diffusion more rapidly than do animals 18 days of age or older. To learn whether the saturable absorption process for DSCG is present at 1 day of age, or whether it appears later during postnatal development, the percentage of a dose of DSCG absorbed by the saturable process in 35 min was calculated for rats of various ages by subtracting the diffusion component of absorption from the overall percentage absorption (Table III) (4). In rats 1–3 days of age, absorption of the compound by saturable transport amounted to 11.6–16.2% of the dose, whereas in animals 6 days of age or older transport was two to three times greater, 34.1–42.9% of the dose.

Discussion. Disodium cromoglycate (DSCG), a drug used clinically by inhalation, appears to be absorbed from the 6-day-old rat lung in part by diffusion and in part by a saturable, carrier-type transport process which qualitatively resembles that previously reported for adult animals (4). For example, in neonatal rats, as the concentration of DSCG is progressively increased, the percentage absorption decreases until the carrier component of absorption becomes saturated at a concentration of about 25 m*M*. While a similar pattern has been reported in adult rats (4), saturation occurs at a higher concentration, about 50 m*M*. Further evidence for carrier-mediated transport of DSCG in the neonatal rat lung was provided by the finding that certain other organic anions, such as phenol red, benzylpenicillin, and *p*-aminohippuric acid, depress the rate of absorption of DSCG. Results similar to these were reported previously for adult rats except that *p*-aminohippuric acid, studied at lower concentrations than in the present work, did not show significant inhibition of DSCG transport. It is of interest to note that, in the adult

rat lung, phenol red has been shown to be absorbed by the same carrier-mediated process that transports DSCG (4, 9), whereas *p*-aminohippuric acid (10) and benzylpenicillin (11) appear to be absorbed by diffusion alone. Therefore, the present finding of an interaction of these anionic compounds with the carrier process for DSCG does not necessarily indicate that all three are absorbed by the carrier process in neonatal rats.

The proportion of DSCG that is absorbed from the rat lung by simple diffusion varies with the age of the animal. For example, at a high drug concentration (50 m*M*), at which diffusion predominates as the mechanism of absorption, it was seen that rats 1–12 days of age absorbed DSCG roughly twice as rapidly as rats 18 days of age or older (Table III). This result is in accord with a previous study which showed that a number of other lipid-insoluble compounds are absorbed by diffusion approximately two times faster in rats 12 days of age or younger than in animals 18 days old or older. Since lipid-soluble drugs do not show this age-related change in absorption rate, the results with lipid-insoluble compounds have been explained in terms of a decrease in membrane porosity which occurs around the age of 15 days (1, 12).

The carrier-mediated absorption process for DSCG is present in the lungs of rats as young as 1 day of age, but the process does not appear to become quantitatively mature until an age of approximately 6 days when the transport rate is two to three times greater than that seen at the ages of 1–3 days.

Summary. Disodium cromoglycate (DSCG) is absorbed from the lungs of the 6-day-old rat in part by a carrier-type transport process and in part by diffusion. The carrier process is saturated at DSCG concentrations greater than 25 m*M* and is inhibited by certain other organic anions including phenol red, benzylpenicillin, and *p*-aminohippuric acid. Although the carrier-mediated absorption process is present in the lungs of rats as young as 1 day of age, the process does not appear to become quantitatively mature until an age of 6 days when the transport rate is two to three times greater than that seen at the age of 1–3 days.

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