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Prenatal induction of Na,K-stimulated adenosine 5′-triphosphatase activity in hamster intestine

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Na,K-stimulated adenosine triphosphatase (EC 3.6.1.3) is found in the surface membranes and the brush borders of microvilli in animal cells. Recent studies indicate that high levels of ouabain-sensitive Na,K-ATPase activity have been isolated from fractions containing the basal and lateral plasma membranes of the intestinal epithelium [1]. Crane [2] documented evidence that the "sodium pump" mechanism was situated at a locus lateral to the plasma membranes in the brush borders and that Na,K-ATPase was an intricate part of the mechanism. In intestinal tissue, especially in the mucosal cells, Na,K-ATPase is recovered in high yields and can be measured easily from homogenates of a whole intestine or isolated mucosa [3,4]. Furthermore, intestinal Na,K-ATPase can be induced in young adult rats by steroids, gluco and mineralcorticoids [5], and by dietary adaptation [4].

This study shows the inducibility of Na,K-ATPase activity by carcinogenic compounds and further characterizes the subsequent altered state of the enzyme under these conditions. Hydrazine and 1,2-dimethylhydrazine are two environmentally hazardous compounds that are used as missile propellants. They are active components in jet fuel exhausts and have been considered a major concern of Air Force propellant toxicological research for many years [6]. Much recent work has centered around the effects of hydrazine and its methylated derivatives on the central nervous system. They have been shown to have toxic effects on amphibia and, in solution, these compounds caused teratogenic effects in *Xenopus laevis* embryos [7,8]. Hydrazine metabolites have been shown to produce colorectal cancer. 1,2-Dimethylhydrazine produced tumors in rats after subcutaneous or oral administration [9,10]. Evidence has been presented indicating that exposure of fetal rats to hydrazine produced neoplastic growths in the jejunum and brain [11]. Hydrazine and 1,2-dimethylhydrazine produced significant effects on the development of the brush border enzymes, lactase, sucrase and alkaline phosphatase, in hamster intestine [12].

In the present study, prenatal effects of hydrazine and 1,2-dimethylhydrazine were examined in fetal hamsters aged 15 days of gestation and monitored during the neonatal and postnatal stages of development to establish long-term effects on Na,K-ATPase levels. Comparisons were made with normal intestines, and possible correlations with the normal metabolic functions of this membranous enzyme are presented.

The hamsters were obtained as pregnant Syrian golden-hamster females, LVG-LAK strain, from Charles River/Lakeview, Wilmington, MA, U.S.A. The animals were specific-pathogen free and were fed *ad lib.* on Wayne Sterilizable Lab-Blox. The animals were dated from the time of observed breeding, assuming a 16-day gestation period. On day 12, the hamsters were assigned randomly to different groups, weighed, and injected. On day 15 (1 day before birth), pregnancy was terminated (N = 4 for each group per day) by decapitation of the mother. Fetuses were extracted after caesarian section. Fetuses from each dam were pooled as one sample. After removal, each fetus was counted, cleaned and weighed. They were then decapitated (all heads were saved in Bouin's solution for teratogenic observation), and intestines were removed, pooled from each litter and then kept cold in beakers on ice. Intestinal homogenates were prepared from whole intestines as a 10 per cent (w/v) solution using a Potter–Elvehjem homogenizer in 0.3 M Tris buffer, pH 7.4. Assays for Na,K-ATPase activity were performed within 2 hr after collection of tissue. Fetal hamsters from each group not used in prenatal experiments were allowed to grow and were delivered normally. Neonates from each group of litters were counted and decapitated (heads from all litters of newborns were saved for observation), and intestinal tissue was processed the same as for the fetal tissue. The sizes of the litters and individual weights of the pups were no different in each of the groups throughout the time studies.

Hydrazine hydrate (85 per cent purified solution, Fisher Scientific Co., Fairlawn, NJ. U.S.A.) and 1,2-dimethylhydrazine dihydrochloride (97 per cent, Aldrich Chemical Co., Milwaukee, WI, U.S.A.) were injected intramuscularly at doses of 15 mg/100 g body wt and 20 mg/100 g body wt respectively. Controls were injected with doses of saline solution. All previously named solutions were prepared in doubly distilled water.

Ouabain-sensitive Na,K-ATPase activity was assayed in a reaction mixture containing 2 mg of intestinal homogenate, 6.7 mM ATP and 2.8 ml buffer (0.03 M Tris, 0.1 M NaCl and 0.01 M KCl, pH 7.4) in the presence and absence of 2.1 mM ouabain (Boehringer-Mannheim, New York, NY, U.S.A.) in a final volume of 3.0 ml [3]. Exogenous additions of Mg (final concentration of 7.5 mM) were without effect on the assay when the whole homogenate was used as the enzyme source. Samples were incubated for 20 min at 37° after which the reaction was terminated by the addition of 1.5 ml of 10 per cent trichloroacetic acid (Fisher Scientific Co., Fairlawn, NJ. U.S.A.). Under these optimal conditions, the reaction was linear with time and protein concentrations up to 3 mg of protein per assay flask. The liberated inorganic phosphate was determined with the Fiske–Subbarow reagent [13]. In addition, a stan-

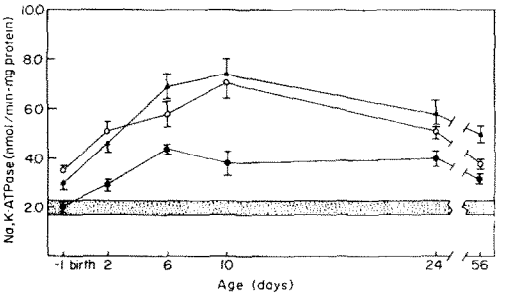

Fig. 1. Induction of Na,K-ATPase activity from control, hydrazine-treated and 1,2-dimethylhydrazine-treated animals of varying developmental age. Each point represents the mean ± S.E.M. of four separate determinations. The specific activity is expressed as nmoles/min·mg of intestinal protein. The stippled overlay indicates the mean ± S.E.M. for Na,K-ATPase activity in the adult hamster (65 day), determined in four male and four female intestinal preparations. Key: (●) control; (▲) hydrazine-treated; and (○) 1,2-dimethylhydrazine-treated.

Table 1. Effects of hydrazine and 1,2-dimethylhydrazine on Na,K-ATPase activities in total homogenates of fetal, neonatal and adult hamster intestines*

	Fetal	Neonatal (sp. act.)	Adult
Control	2.0 ± 0.3†‡§	3.8 ± 0.4‡§‖	2.1 ± 0.1§
Hydrazine	3.0 ± 0.1†‖	7.4 ± 0.5‖	2.3 ± 0.2§
1,2-Dimethyl hydrazine	3.5 ± 0.1†	7.0 ± 0.5‖	3.4 ± 0.3

* Values are given in nmoles/min·mg total protein and are expressed as the mean ± S.E.M. for the determinations on four separate preparations from −1 day, 10-day, and adult (65-day) hamster intestine. Symbols †–‖ indicate statistically significant differences ($2P < 0.05$) based on the two-sided U-test.

† Different from neonatal.

‡ Different from hydrazine-treated animals.

§ Different from 1,2-dimethylhydrazine-treated animals.

‖ Different from adult.

dard curve was run with each experiment using sodium phosphate. All specific activities were expressed as nmoles of phosphorus liberated/min·mg of intestinal protein. Total protein concentrations in the intestinal homogenates were determined by the procedure of Lowry *et al.* [14], with bovine serum albumin (essentially fatty acid-free, Sigma Chemical Co., St. Louis, MO, U.S.A.) as the standard.

The intestinal developmental patterns of Na,K-ATPase activities from control, hydrazine and 1,2-dimethylhydrazine-treated hamsters are presented in Fig. 1. Throughout the range of −1 day to 24 days of age, Na,K-ATPase activities in the treated groups increased significantly in comparison to the control groups. Throughout these studies there was no statistically significant difference, $2P < 0.05$, between the specific activities of the adult male and female intestinal preparations. The adult value presented represents the means of eight separate determinations, four with each sex. The general appearance of the offspring in all groups was not affected, i.e. litter size, weight and resorptions were minimal. Heads from all litters were screened for teratogenic effects. No abnormalities were found in the control animals or in either of the treated groups. Prenatal exposure to hydrazine and 1,2-dimethylhydrazine altered the normal developmental pattern of Na,K-ATPase activity. Elevation of the levels, in comparison to controls, was evident through the fetal, neonatal and adult stages. The neonatal peak levels in the hydrazine and 1,2-dimethylhydrazine-treated animals occurred at the 10-day postnatal period. Fetal levels in the treatment groups were elevated in comparison to the normal adult levels, whereas the control level was approximately the same as the adult level during the fetal stage. The peak level for Na,K-ATPase activity in the control group was evident at the 6-day postnatal period; however, this value decreased to a value near the adult level at the 56-day period of development (Fig. 1).

The results in Table 1 summarize data concerning the specific activities of Na,K-ATPase with control, hydrazine and 1,2-dimethylhydrazine-treated animals at three stages of development. As presented in Table 1, the fetal and neonatal values in both the hydrazine and the 1,2-dimethylhydrazine-treated animals are significantly greater than the fetal and neonatal values in the control group. The 1,2-dimethylhydrazine-treated adult level of Na,K-ATPase activity is higher than both the hydrazine-treated and the control adult levels.

The data demonstrate that the activity of Na,K-ATPase can be enhanced prenatally in hamster intestine by hydrazine and 1,2-dimethylhydrazine. The stimulatory effect took place in less than 3 days after administration and continued through the fetal, neonatal and adult stages. The mechanism by which this effect occurs is not known. Transplacental passage of carcinogenic compounds such as methylazoxymethanol, an active metabolite of 1,2-dimethylhydrazine, has been demonstrated in the hamster by thin-layer chromatography [15]. A direct effect on fetal tissues, therefore, is possible; however, changes in maternal concentrations of Na,K-ATPase could affect fetal tissues indirectly. This may be important in the pathogenesis of a malformation or tumor formation during intrauterine life.

The present study has demonstrated that Na,K-ATPase is responsive to prenatal enhancement and that fetal intestinal cells are capable of reacting to a stimulation by hydrazine and 1,2-dimethylhydrazine. Altered enzyme levels are being used as indicators of toxicity in general toxicology studies [12,16–18]. This study has examined altered enzyme levels in a specific organ after prenatal exposure to a carcinogen specific for that organ. The results indicate that prenatal exposure to hydrazine and 1,2-dimethylhydrazine does evoke a significant alteration in the normal pattern of intestinal Na,K-ATPase development.

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REFERENCES

1. M. Fujita, H. Ohta, K. Kawai, H. Matsui and M. Nakao, *Biochim. biophys. Acta* **274**, 336 (1972).
2. R. K. Crane, in *Intestinal Absorption and Malabsorption* (Ed. T. Z. Csaky), p. 127. Raven Press, New York (1975).
3. J. P. Quigley and G. S. Gotterer, *Biochim. biophys. Acta* **255**, 107 (1972).
4. H. Schiffel and K. Loeschke, *Pflügers Arch. ges. Physiol.* **372**, 83 (1977).
5. A. N. Charney, M. D. Kinsey, L. Myers, R. A. Giannella and R. E. Gots, *J. clin. Invest.* **56**, 653 (1975).
6. K. C. Back and A. A. Thomas, *A. Rev. Pharmac.* **10**, 395 (1970).
7. G. Greenhouse, in *Environmental Quality Research*, National Technical Information Service, Reprint AMRL-TR 74-82, p. 49. National Technical Information Service, Washington, DC (1975).
8. G. Greenhouse, *Teratology* **13**, 167 (1976).
9. H. Druckery, in *Fortschritte det Krebsforchung* (Eds. C. G. Schmidt and O. Wetter), p. 37. F. K. Schattaur, Stuttgart (1969).
10. H. Druckery, in *Carcinoma of the Colon and Antecedent Epithelium* (Ed. W. J. Burdette), p. 267. C. C. Thomas, Springfield, IL (1970).
11. M. Spatz, *Ann. N.Y. Acad. Sci.* **163**, 848 (1969).

12. C. M. Schiller, R. Walden and T. E. Kee, *Toxic. appl. Pharmac.* **49**, 305 (1979).
13. C. H. Fiske and Y. Subbarow, *J. biol. Chem.* **66**, 375 (1926).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. M. Spatz and G. L. Lacquer, *Proc. Soc. exp. Biol. Med.* **127**, 281 (1968).
16. G. W. Lucier, B. R. Sonawane, O. S. McDaniel and G. E. R. Hook, *Chem. Biol. Interact.* **11**, 15 (1975).
17. B. A. Fowler and J. S. Woods, *Lab. Invest.* **36**, 122 (1977).
18. C. M. Schiller and G. W. Lucier, *Chem. Biol. Interact.* **22**, 199 (1978).

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Prediction of mescaline clearance by rabbit lung and liver from enzyme kinetic data

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Although many organs metabolize drugs and other xenobiotic agents, the liver is commonly accepted as the primary organ of drug metabolism *in vivo* since hepatic enzyme content is usually much greater than that of other organs. It has become evident, however, that enzyme content is not the sole determinant of drug clearance. Substrate delivery to a clearing organ, determined in part by the blood flow to the organ and by the extent of binding of the drug to plasma proteins, is also important [1].

In the rabbit, mescaline clearance is due to metabolism by an amine oxidase, the amount of which is much greater in liver than in other organs [2]. Although the lung contains less enzyme than liver, it receives the entire cardiac output while liver blood flow is only one-fourth of this value [3–6]. The higher blood flow to the lungs and, hence, greater substrate delivery may permit the lungs to contribute significantly to the clearance of mescaline *in vivo*.

In the isolated, perfused liver [7] as well as *in vivo* [5,6,8], clearance of drugs which are eliminated by hepatic metabolism has been shown to depend upon hepatic blood flow in the following manner:

$$Cl = \frac{Qf_B Cl_{int}}{Q + f_B Cl_{int}}, \quad (1)$$

where Q in the blood flow to the eliminating organ, f_B is the fraction of drug unbound in the plasma, and Cl_{int} is the intrinsic metabolic clearance. At low flows, clearance, as defined in equation (1), is proportional to flow; that is, substrate delivery limits clearance. At high flows, clearance approaches the intrinsic metabolic clearance, which is the maximum clearance of which an organ is capable when drug delivery is not limiting. Rane *et al.* [7] have shown that the intrinsic clearance of drugs eliminated by hepatic metabolism can be predicted from enzyme kinetic parameters using the following relationship:

$$Cl_{int} = \frac{V_{max}}{K_m}, \quad (2)$$

where V_{max} is the maximum rate of metabolism and K_m is the apparent Michaelis–Menten constant of the enzyme responsible for disposition.

We have extended the above model to predict pulmonary drug clearance. Since mescaline is cleared rapidly by isolated rabbit lungs perfused at low flows [2], it was of interest to predict the role of lung relative to liver in the clearance of mescaline *in vivo*. In the present study we have determined the kinetic constants for mescaline oxidase activity *in vitro* and have used the model of Rane *et al.* [7] to predict the relative clearance by rabbit lung and liver *in vivo*.

Female rabbits (3–4.5 kg) were given 5000 units of heparin i.v. and anesthetized by subsequent administration of pentobarbital (30 mg/kg, i.v.); the organs were cleared of blood as described previously [2]. The liver and lungs were then removed, weighed and homogenized in 0.1 M phosphate buffer containing 0.25 M sucrose (pH 7.4) in a Waring Blendor. Lungs and livers were homogenized in 12 and 6 vol. of buffer, respectively, and the homogenates were centrifuged for 10 min at 600 *g*. The resulting supernatant fractions were used in determining mescaline metabolism. Protein was analyzed by the method of Lowry *et al.* [9].

Determination of mescaline oxidase activity has been described previously [2]. Briefly, reaction mixtures contained 1 μM [8–^{14}C]mescaline hydrochloride (22.8 mCi/mmole, New England Nuclear, Boston, MA), sufficient cold mescaline hydrochloride (Aldrich Chemical Co., Milwaukee, WI) to achieve concentrations of 25, 50, 75, 100, 125 or 200 μM, 0.8 ml of phosphate buffer and 0.2 ml of the 600 *g* supernatant fraction of liver or lung homogenates. Reaction mixtures were incubated for 10 min at 37°. The reaction was stopped by the addition of 0.2 ml of 0.2 M $ZnSO_4$ followed by 0.2 ml of 0.2 M $Ba(OH)_2$. The

Table 1. Mescaline oxidase kinetics in 600 *g* supernatant fractions of rabbit lung and liver homogenates

Organ	K_m* (μM)	V_{max}* (nmole/min) (per g protein)	(per g organ)	(per organ)	Predicted Cl_{int}† (ml/min)
Lung	41.1 ± 10.9	739 ± 193	142 ± 36	1847 ± 432	56 ± 17
Liver	48.7 ± 6.1	351 ± 9	144 ± 8	13,750 ± 1,784	282 ± 56
Lung/liver	0.84	2.11	0.99	0.13	0.20

* Values represent means ± S.E.M. for five animals. Mescaline oxidase kinetic constants were determined as described in the text.

† Intrinsic metabolic clearance was calculated as $Cl_{int} = V_{max}/K_m$.