0031-3998/88/2304-0414$02.00/0
PEDIATRIC RESEARCH

Vol. 23, No. 4, 1988
Printed in U.S.A.

Comparison of the Digitalis Receptor in Erythrocytes from Preterm Infants and Adults

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ABSTRACT. We compared *86*rubidium by erythrocytes of preterm infants and adults as a measurement of their Na^+, K^+, ATPase enzyme system. In neonates, total uptake (0.92 ± 0.13 μg/10^6 cells) and specific uptake (0.64 ± 0.076 μg/10^6 cells) were significantly higher than in adults (0.52 ± 0.1 and 0.29 ± 0.06 μg/10^6 cells, respectively; $p < 0.025$). The percentage of specific uptake from total uptake was higher in infants (73.3 ± 2.3%) than in adults (57.9 ± 4.6%) ($p < 0.005$). No differences were found in the affinity constant of ^{86}Rb uptake between infants (4.35 ± 0.48 ng/ml) and adults (4.85 ± 0.48 ng/ml). Stratification of infants according to their serum K^+ concentrations revealed that levels above 5.4 mEq/liter were associated with a higher specific uptake (0.79 ± 0.107 μg/10^6 cells) than in normokalemic infants (0.54 ± 0.09 μg/10^6 cells) or adults (0.304 ± 0.061 μg/10^6 cells) ($p < 0.05$). The difference between hyperkalemic and normokalemic infants persisted after excluding those who received adult packed cells (0.88 ± 0.1 and 0.6 ± 0.12 μg/10^6 cells, respectively) ($p < 0.05$). Infants with serum $K^+ > 5.8$ mEq/liter received on average significantly more K^+ in previous days (2.46 ± 0.49 *versus* 1.13 + 0.34 mEq/kg·day; $p < 0.025$). The different K^+ level could not be attributed to different creatinine clearance in the two groups. (*Pediatr Res* 23:414–417, 1988)

Abbreviations

Rb, rubidium
RBC, red blood cells
Bmax, total specific uptake capacity

Despite continuous controversy over their clinical efficacy, digitalis glycosides are still one of the most commonly prescribed group of drugs (1). In infants and children, only a few controlled studies assessed the effect of digoxin in heart failure associated with congenital heart defects, and their results are inconclusive (2). Pharmacokinetic analysis has revealed that infants and children need higher doses per kg of digoxin than adults to achieve comparable serum concentrations due to faster clearance rates (3). However, in some cases these differences are minimized by calculation of dose according to surface area.

The hypothesis that the immature organism is less sensitive to digoxin than the adult, both in terms of pharmacologic and toxicologic effects, has been based on animal studies (4–6) but never proven in infants and children.

Received July 27, 1987; accepted December 14, 1987.
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Supported by Grant MS8544 of the Medical Research Council of Canada. G.K. is supported by a Career Scientist Scholarship of Ontario Ministry of Health.

The membrane Na^+, K^+, ATPase is considered by most authorities to be the pharmacologic receptor for digitalis glycosides (7). By specifically inhibiting this enzyme, higher intracellular concentrations of sodium are achieved; exchange of increased amounts of sodium with more extracellular calcium results in increased cardiac ionotrophy (8). However, the mechanism of action of digitalis in increasing cardiac inotrophy is still largely conjectural.

Rb is a kaliumimetic cation that moves across cell membranes in a fashion similar to potassium (9). Thus, by measuring Rb^+ uptake, one may assess the activity of the membrane Na^+, K^+, ATPase enzyme both in terms of binding capacity and affinity (10).

Herein we compared the digitalis receptor between preterm infants and adults. Specifically, we wished to identify within the group of infants the association between serum electrolytes and binding capacity of the receptor.

MATERIALS AND METHODS

Patients. This protocol was approved by the Hospital's Committee on Human Experimentations. Our patients were 31 newborn infants (27 preterm, gestational age 25–37 wk, mean ± SEM 29 ± 0.8 wk; four term). Their postnatal age was between 2–57 days (mean 12.4 ± 2.9).

All were hospitalized in our neonatal ICU because of prematurity, suspected sepsis, or respiratory distress syndrome. Twelve healthy adult volunteers served as a second group for the ^{86}Rb uptake studies.

Clinical studies. At the day of the study 1-ml heparinized blood samples were drawn from an existing intravenous indwelling catheter for ^{86}Rb uptake studies. Electrolytes and creatinine were determined in the serum and in 6–8 h urine collection. Creatinine clearance and fractional excretion of sodium and potassium were determined using standard methods. In each infant, potassium intake was calculated for the 2 days before the study and during the day of the study.

^{86}Rb uptake studies. For the uptake assay of Rb in RBC the method described by Aronson *et al.* (11) was used with some modifications.

Erythrocytes were separated by centrifugation and washed three times in a potassium-free Ringer solution. Subsequently, they were diluted 1:3 with potassium-free Ringer solution and 100 ml of this solution was incubated with ouabain (concentrations between 0–50 ng/ml) in a total volume of 300 μl at 37° C for 2 h.

At the end of this step, 100 μl of K-free Ringer solution containing ^{86}Rb (New England Nuclear Ltd., 10 mCi/ml, 4.78 mCi/mg) diluted with cold Rb to a final concentration of 23 μg/ml, was added to each tube and the incubation continued for

another hour. Specific activity was corrected according to the decay half-life of the labeled ^{86}Rb.

At the end of the incubation 50-μl aliquots were added in duplicate to a conical 1.5 ml tube in which 0.8 ml 110 mM $MgCl_2$ was layered over 0.6 ml dibutyl phtalate. The tubes were centrifuged for 20 s at low speed (2,000 rpm) and for 40 s at high speed (10,000 rpm). The supernatant was aspirated and then the bottom of the tubes, with the RBC, was cut and transferred into scintillation vials containing 1 ml of isopropanol:toluene 1:1. After 30 min, 15 ml of scintillation cocktail (Hionic-fluor) was added and the vials were counted in a Beckman Counter for 2 min. The uptake is expressed as μg Rb/10^6 cells. The RBC were counted in a Coulter counter using the working RBC solution.

Calculations. Specific uptake of ^{86}Rb was determined as a total uptake minus uptake in the presence of excess ouabain (50 ng/ml). Preliminary studies revealed that uptake in the presence of 50 ng/ml ouabain is similar to uptake in the presence of 1 μg/ml of ouabain. Figure 1 shows a typical curve of specific ^{86}Rb uptake *versus* concentration of ouabain. Each curve was fitted to an exponential term using the MACFIT computer program (Tesseract Educational Systems Ltd). From this curve the affinity constant of ouabain to ^{86}Rb was calculated (concentration of ouabain at which 50% of specific ^{86}Rb uptake is inhibited). In addition, the total specific uptake capacity of ^{86}Rb (Bmax) was derived. Total (specific plus nonspecific) ^{86}Rb uptake was calculated as uptake in the absence of ouabain.

Correlation between different parameters (see "Results") were studied using the MACFIT program. Both linear and nonlinear equations were tested. Differences between means of two groups were compared by Student's t test for unpaired results. Differences between means of more than two groups were compared by analysis of variance and Duncan's multiple range test. Results are expressed as mean ± SEM.

RESULTS

Total ^{86}Rb uptake was significantly higher in infants (0.92 ± 0.132 μg/10^6 cells) than in adults (0.52 ± 0.1 μg/10^6 cells) ($p < 0.05$). Similarly, specific ^{86}Rb uptake was significantly higher in the infants (0.64 ± 0.076 μg/10^6 cells and 0.29 ± 0.061, respectively, $p < 0.025$). The percentage of specific uptake from the total uptake was higher in infants (73.3 ± 2.3 *versus* 57.9 ± 4.6%, $p < 0.005$). No differences were found in the affinity constant of ^{86}Rb uptake (Kd) between adults (4.3 ± 0.67 ng/ml) and infants (4.85 ± 0.48 ng/ml). Within the neonatal group, no correlation could be found between gestational or postconceptual age and ^{86}Rb total of specific uptake.

In order to study a possible association between ^{86}Rb uptake and serum potassium concentrations, we stratified the neonates into two groups, with an arbitrary cutting point of 5.4 mEq/liter.

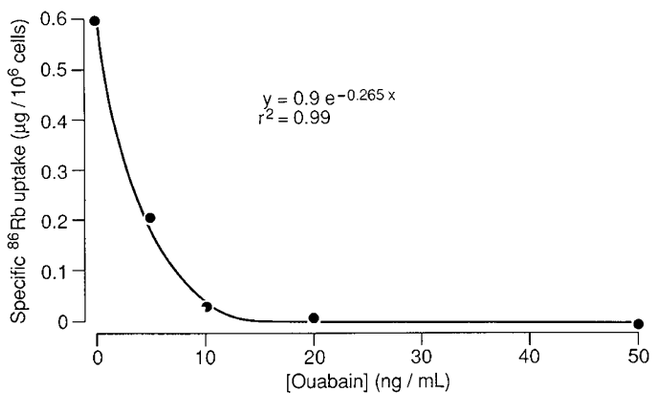

Fig. 1. A typical curve of specific ^{86}Rb uptake *versus* concentration of ouabain.

Infants and normokalemia (range 3.6–5.4 mEq/liter) ($n = 19$) had ^{86}Rb uptake significantly higher than adults (0.54 ± 0.166 *versus* 0.29 ± 0.061 μg/10^6 cells, respectively) ($p < 0.05$). Infants with K^+ higher than 5.4 mEq/liter (range 5.8–7.6) ($n = 12$) had Bmax of 0.79 + 0.107 mg/10^6 cells, significantly higher than adults ($p < 0.01$) or infants with normokalemia ($p < 0.05$) (Fig. 2). Hyperkalemia was not associated with different Kd of rubidium uptake. Four normokalemic and two hyperkalemic infants received transfusions of packed cells before the study day in a total of less than 10% of their blood volume. To correct for possible effect of adult erythrocytes, the calculation was repeated after excluding them, again showing higher specific binding of ^{86}Rb in hyperkalemia (0.88 ± 0.1 *versus* 0.6 ± 0.12 μg/10^6 cells, $p < 0.05$).

Infants with serum K^+ higher than 5.4 mEq/liter received on average significantly more K^+ during the 2 and 1 days before and the day of the study (2.46 ± 0.45 *versus* 1.13 ± 0.34 mEq/kg·day; $p < 0.025$). Infants with hyperkalemia had creatinine clearances not significantly different from the normokalemic babies (23.35 ± 4.7 *vs* 34.8 ± 6.1 ml/kg h; $p > 0.1$). The two groups had a similar postconceptional age (224 ± 15.2 and 227.3 ± 8.9 days, respectively) when studied. The two groups were similar in their clinical conditions; most infants were ventilated, and under ventilation none of the infants had hypoxia or acid based imbalance (Table 1). Significant positive correlation existed between serum potassium concentrations and daily excretion of K^+ (Fig. 3) and between daily potassium intake and urinary excretion of the cation (Fig. 4). No correlation could be found between serum potassium concentrations and fractional excretion of potassium or sodium.

DISCUSSION

A variety of animal studies has documented less sensitivity of the newborn to digitalis when compared to adults (4, 5, 12). Achieving either clinical or toxic effects of digitalis requires larger doses of the glycosides in the immature animal. Such discrepancy can be partially explained by a higher clearance rate of digoxin during development; however, in several studies the lesser sensitivity to the glycoside could be demonstrated even when serum concentrations were maintained at similar levels in young and adult animals (4). No such studies are available in humans and it is highly questionable whether higher doses are needed in infants to achieve a clinical response.

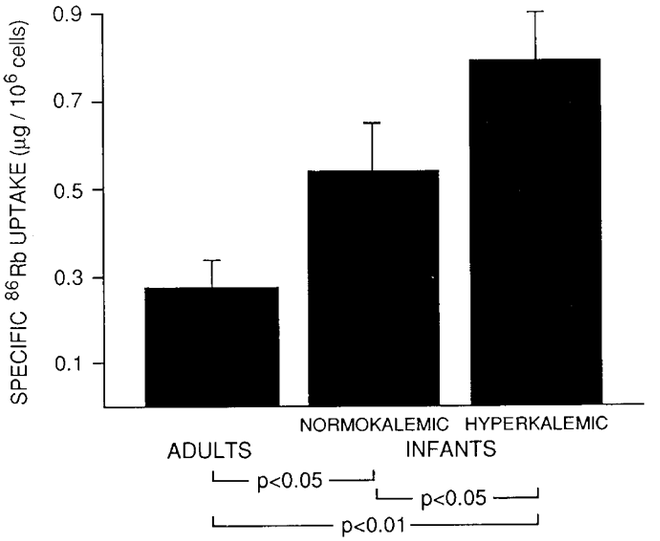

Fig. 2. Total specific ^{86}Rb uptake by erythrocytes of adults and preterm infants stratified by serum K^+ concentrations. Hyperkalemic infants had significantly higher uptake than normokalemic babies, who in turn, had significantly higher uptake than adults.

Table 1. *Clinical characteristics of infants with normokalemia (≤5.4 mEq/liter vs hyperkalemia (>5.7 mEq/liter)*

	Normokalemia	Hyperkalemia	Significance
n	19	12	
Range of serum K^+ (mEq/liter)	3.6–5.4	5.8–7.6	
Mean ± SD of serum K^+ (mEq/liter)	4.76 ± 0.11	6.35 ± 0.15	$p < 0.0001$
Postconceptional age (day)	224 ± 15.2	227.3 ± 8.9	NS
Creatinine clearance (ml/kg·h)	23.35 ± 4.7	34.8 ± 6.1	NS
Serum Na^+ (mEq/liter)	137.4 ± 2.1	136.9 ± 1.5	NS
Average dose of K^+ in preceding days (mEq/kg·day)	1.13 ± 0.34	2.46 ± 0.45	$p < 0.025$
Term/preterm	2/17	2/10	NS
Ventilated	16/19	10/12	NS
Hypoxic (<60 mm Hg on ventilator)	0/19	0/12	NS
Acidosis (pH <7.25)	0/19	0/12	NS

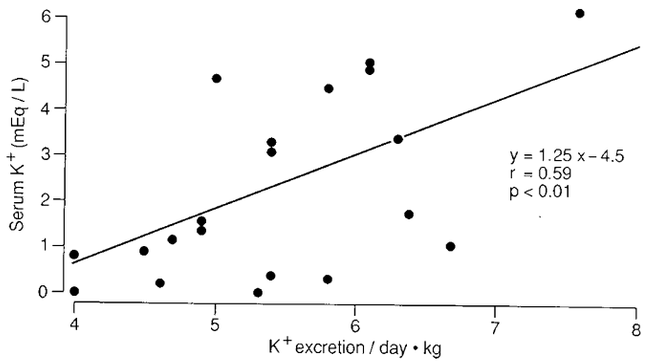

Fig. 3. Correlation between serum potassium concentrations at the day of the study and daily excretion of K^+ in the days 0–2 before the study in newborn infants.

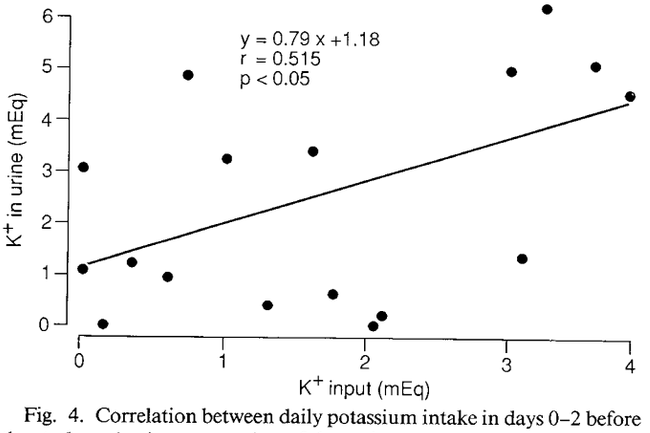

Fig. 4. Correlation between daily potassium intake in days 0–2 before the study and urinary excretion of the cation.

Although the membrane Na^+, K^+, ATPase is generally accepted as the cardiac pharmacologic receptor of digitalis (7), several inconsistencies have been recognized in recent years. For example, positive ionotrophy could be shown to persist long after measurable inhibition of Na^+, K^+, ATPase has subsided (8). This points to the fact that the true mechanism of digoxin's positive inotropic effect is still largely unknown. By measuring Na^+, K^+, ATPase activity in a peripheral cell such as the erythrocyte, one makes the assumption that this receptor represents the events occurring at the same receptor in the cardiac muscle. Several human studies in the last decade could show a significant positive correlation between inhibition of erythrocyte Na^+, K^+, ATPase and digoxin serum concentrations (11) as well as changes in systolic time interval (10).

Our results indicate a significantly higher ^{86}Rb uptake by erythrocytes of preterm infants when compared to adults. Both the total binding and the specific (ouabain inhibitable) binding are higher in the preterm infant. A higher binding capacity of the digitalis receptor may mean that more molecules of the glycoside are needed to cause a pharmacologic or toxic effect. These data agree with previous animal and human studies (13–15) that investigated umbilical cord blood without mentioning gestational age. Similar to our results, Kelly *et al.* (13) could not detect a significantly different affinity in the ^{86}Rb uptake of infants' erythrocytes when compared to their mothers. The same group, in another study, reported different dissociation constants for infants and adults (15). This time, the adults had a significantly lower value when compared to the neonates. The differences in results among Kelly *et al.* (13) and ours, on the one hand, to Kearins *et al.* (15), on the other hand, may be attributed to the different methods used. Similar to our data Kelly *et al.* (13) studied ^{86}Rb uptake whereas Kearin *et al.* (15) report on the binding of tritiated ouabain to cell membranes. Presently, it is not known whether the presence of fetal hemoglobin in neonates affect rubidium uptake, and thus yielding differences between ^{86}Rb uptake and membrane ATPase studies.

In an attempt to identify possible determinants of higher density of Na^+, K^+, ATPase we stratified our infants according to their serum potassium and showed that preterm infants with serum potassium above 5.7 mEq/liter had significantly higher specific ^{86}Rb uptake when compared to normokalemic infants. Both groups of infants had higher ^{86}Rb uptake than healthy adults.

Our hyperkalemic infants received significantly more potassium than the normokalemic. It is possible that the sustained hyperkalemia results in up-regulation of the receptor as well as other potassium pumps in a compensatory effort to lower serum potassium levels.

It is possible that preterm infants are different from the full term in the Na^+, K^+, ATPase density. Other studies in the newborn infant (13, 15) did not specify maturity of potassium levels. Moreover, these authors did not correct their assay for cell numbers and/or hematocrit, but rather used a given volume of washed RBC. The newborn is known to have a larger mean corpuscular volume and larger surface area (16) than the adult, and this may partially account for higher Bmax when compared to adults.

In contradiction to animal studies, the notion that infants are less sensitive to digoxin effects and toxicity has not been proven. In most cases, infants with congestive heart failure are not treated only by digitalis; it is therefore difficult to directly assess the effect of the glycoside. In a study where only digoxin was used, about 50% of infants and small children with ventricular septal defect appeared to improve clinically with digoxin serum levels of 1.5 ng/ml (2). Inhibition of erythrocyte ^{86}Rb uptake did not differ between responders and nonresponders.

Our preliminary observation of possible effect of hyperkalemia on ^{86}Rb uptake calls for controlled animal studies where different levels of hyperkalemia will be induced through increased intake of the cation. In addition, it would be important to compare under the same conditions newborn infants of various gestational ages in order to assess the developmental aspects of this receptor. Finally, more studies are needed in infants and children to correlate positive ionotrophism with the degree of inhibition of Na^+, K^+, ATPase at a peripheral cell such as the erythrocyte.

REFERENCES

1. Tallardia RJ 1985 Most prescribed drugs 1985. Philadelphia, WB Saunders
2. Berman WJ, Yabek SM, Dillon T, Niland C, Carlew S, Christensen D 1983 Effect of digoxin in infants with a congested circulatory state due to a ventricular septal defect. N Engl J Med 308:363–366
3. Gorodischer R 1980 Cardiac drugs. In: Yaffe SJ (ed) Pediatric Pharmacology, Grune & Stratton, Inc., New York, pp 305–328
4. Berman WJ, Musselman J, Shortencarrier R 1980 The physiological effects of digoxin under steady state drug conditions in newborn and adult sheep. Circulation 62:1165–1171
5. Rosen KG, Sigstrom L 1978 The influence of age on Na^+, K^+, ATPase activity in erythrocytes in fetal and newborn guinea pigs. J Perinatol Med 6:154–159
6. Berman W Jr, Ravenscroft PJ, Sheiner LB, Heymann MA, Melmon KL, Rudolph AM 1977 Differential effects of digoxin at comparable concentrations in tissues of fetal and adult sheep. Cir Res 41:635–642
7. Schwartz A 1974 Is the cell membrane Na^+, K^+, ATPase enzyme system the pharmacological receptor for digitalis? Cir Res 39:2–7
8. Koren G, Soldin SJ 1987 Cardiac glycosides. Lab Med Clin North Am 3:587–606
9. Love WD, Burth GE 1953 A comparison of ^{42}potassium, ^{86}rubidium, and ^{134}cesium as tracers of potassium in the study of cation metabolism of human erythrocytes in vitro. J Lab Clin Med 41:351–362
10. Ford AR, Aronson JK, Graham-Smith DG, Carver JG 1979 Changes in cardiac glycoside receptor sites, ^{86}Rb uptake and intracellular sodium concentrations in the erythrocytes of patients receiving digoxin during the early phases of treatment of cardiac failure in regular rhythm and of atrial fibrillation. Br J Clin Pharmacol 8:129–134
11. Aronson JK, Graham-Smith DG, Hallis KF, Hibble A, Wigley F 1977 Monitoring digoxin therapy: I: Plasma concentrations and an in vitro assay of tissue response. Br J Clin Pharmacol 4:213–221
12. Kelliher GJ, Roberts J 1976 Effect of age on the cardiotoxic action of digitalis. J Pharmacol Exp Ther 197:19–18
13. Kelly JG, McMillen RMC, McDevit DG 1983 The effect of digoxin on ^{86}Rb uptake by erythrocytes from mothers and babies. Br J Clin Pharmacol 15:49–53
14. Marsh AJ, Lloyd BL, Taylor RR 1981 Age dependence of myocardial Na^+, K^+, ATPase activity and digitalis intoxication in the dog and guinea pig. Cir Res 48:329–333
15. Kearin M, Kelly JG, O'Malley K 1980 Digoxin "receptors" in neonates: An explanation of less sensitivity to digoxin than in adults. Clin Pharmacol Ther 28:346–349
16. Weintrobe MM 1985 Clinical Hematology, 9th ed Lea & Febiger, Philadelphia