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Comparative Monovalent Cation Transport in Neonatal and Adult Red Blood Cells

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Summary

Active transport of Na and K under physiologic and maximally stressed conditions was identical in *normal* adult red blood cells (RBC) and term neonatal erythrocytes. These results are consistent with the previous observation that Na-K ATPase is the same in *normal* adult RBC and term neonatal erythrocytes. These data, however, are at variance with a previous observation that active K transport is impaired in neonatal erythrocytes. The most reasonable explanation for this difference relates to inherent problems with the use of radioisotopes which were used in previous *in vitro* studies of cation transport.

Abbreviations

RBC, red blood cells
GBS, glycylglycine-buffered salt solution

It previously has been reported that active K transport is decreased in term neonatal RBC compared to normal adult erythrocytes (1). The explanation for this altered K transport was that neonatal RBC have decreased Na-K ATPase activity (8). Term neonatal RBC do in fact have decreased Na-K ATPase activity compared with adult reticulocyte-rich RBC; however, there is no difference in enzyme activity when compared with normal adult RBC (8). The studies reported here were designed to resolve the paradox of why term neonatal RBC have decreased active K transport despite the fact that they contain the same Na-K ATPase activity as that seen in normal adult RBC.

MATERIALS AND METHODS

General experimental design. Nonisotopic cation fluxes were measured in normal adult RBC and term neonatal erythrocytes. The cation flux studies were determined under normal physiologic conditions and under conditions where the intracellular sodium concentration was elevated to maximally activate Na-K ATPase (2).

Preparation of RBC. Blood was collected in EDTA from the umbilical cords of healthy term infants and from normal adult volunteers. Erythrocytes were separated from the plasma and buffy coat by centrifugation at 4°C. The red blood cells next were washed three times in Na-GBS, pH 7.4 (37°C) with the following composition: 145 mM NaCl, 5 mM KCl, 1 mM Na_2 HPO_4, 1 mM $MgCl_2$, 5 mM glucose, and 20 mM glycylglycine.

Preparation of high sodium RBC. Na-loaded human RBC were prepared by incubating washed RBC (5% hematocrit in Na-GBS) with amphotericin B sulfate at a final concentration of 5 μg/ml. After 2-h incubation in a shaking water bath (100 oscillations/min) at 37°C, RBC were washed five times with 20 volumes of Na-GBS containing bovine albumin (1 g/dl). Albumin was added to bind and remove amphotericin from the cell suspension.

Measurement of RBC cation transport. Red blood cells were suspended in Na-GBS (hematocrit 20%) and incubated 4 h at 37°C in a shaking water bath (100 oscillations/min). Cell Na and medium K were measured every 30 min utilizing previously described methods (3, 4). Changes in cell K were determined indirectly from the hematocrit and medium K concentration. There was no hemolysis during these studies, and thus more precise measurements of net K efflux could be obtained by measuring medium K changes (increased values above a small number). Active transport was defined as the net cation difference between cells incubated in the presence and absence of ouabain (10^{-4} M).

RESULTS

Na-K active transport at low intracellular sodium concentrations. The fresh or "low" Na content of adult and neonatal RBC was maintained during the period of incubation. The mean cell

Na concentration in adult RBC (9.9 ± 0.9 meq/liter RBC) was similar to that in newborn erythrocytes (10.1 ± 1.1 meq/liter RBC) (Table 1). In adult RBC with this low Na content, Na transport out of the cell (1.3 meq/liter RBC/h) was greater than active K transport inwards (1.1 ± 0.2 meq/liter RBC/h), such that the Na:K pump ratio was 1.2 ± 0.1. In newborn erythrocytes, active Na transport (1.2 ± 0.3 meq/liter RBC/h) was similar to that seen in adult RBC as was active K transport (1.0 ± 0.2 meq/liter RBC/h) and the Na:K pump ratio (1.2 ± 0.2).

Na-K active transport at high intracellular sodium concentrations. In RBC preloaded with Na in order to stimulate the Na-K ATPase transport system, the mean cell Na during the incubation period was similar in adult (41.9 ± 3.2 meq/liter RBC) and neonatal (45.2 ± 5.8 meq/liter RBC) erythrocytes (Table 2). Moreover, there was no significant difference in active Na efflux (adult = 3.8 ± 0.6 meq/liter RBC/h, neonate = 3.6 ± 0.7 meq/liter RBC/h), active K influx (adult = 3.0 ± 0.5 meq/liter RBC/h, neonate 2.8 ± 0.7 meq/liter RBC/h) or Na:K pump ratio (adult = 1.3 ± 0.2, neonate = 1.3 ± 0.4).

Table 1. *Active cation transport in adult and neonatal erythrocytes with a low Na content**

	RBC cation change (meq/liter RBC/h)			
	Adult RBC		Neonatal RBC	
	Na	K	Na	K
Without ouabain	+(0.2 ± 0.2)	−(0.3 ± 0.2)	−(0.3 ± 0.2)	−(0.2 ± 0.2)
With ouabain	+(1.5 ± 0.3)	−(1.4 ± 0.3)	+(0.9 ± 0.3)	−(1.2 ± 0.2)
Active transport	1.3 ± 0.2	1.1 ± 0.2	1.2 ± 0.3	1.0 ± 0.2
Na:K pump ratio	1.2 ± 0.2		1.2 ± 0.2	
Mean cell Na (meq/liter RBC)	9.9 ± 0.9		10.1 ± 1.1	

* Fresh adult and cord blood erythrocytes suspended in Na-GBS (hematocrit 20%) were incubated in the presence and absence of ouabain (0.1 mM). Changes in cell sodium (measured directly) and cell potassium (calculated from changes in medium K and hematocrit) were determined every 30 min over a 4-h period. Active action transport was calculated from the difference in net hourly cation change in the presence and absence of ouabain. The sodium/potassium pump ratio was calculated by dividing sodium-active transport by potassium-active transport. Mean cell Na is the average RBC Na content during the period of incubation. Results are expressed as mean ± 1 SD of seven separate experiments.

Table 2. *Active cation transport in adult and neonatal erythrocytes with an elevated Na content**

	RBC cation change (meq/liter RBC/h)			
	Adult RBC		Neonatal RBC	
	Na	K	Na	K
Without ouabain	−(1.9 ± 1.1)	+(0.6 ± 0.5)	−(2.3 ± 0.9)	+(1.2 ± 0.9)
With ouabain	+(1.9 ± 1.2)	−(2.4 ± 0.8)	+(1.3 ± 0.6)	−(1.6 ± 0.6)
Active transport	3.8 ± 0.6	3.0 ± 0.5	3.6 ± 0.7	2.8 ± 0.7
Na:K pump ratio	1.3 ± 0.2		1.3 ± 0.4	
Mean cell Na (meq/liter RBC)	41.9 ± 3.2		45.2 ± 5.8	

* Sodium-loaded adult and cord blood erythrocytes suspended in Na-GBS (hematocrit 20%) were incubated in the presence and absence of ouabain (0.1 mM). Changes in cell sodium (measured directly) and cell potassium (calculated from changes in medium K and hematocrit) were determined every 30 min over a 4-h period. Active cation transport was calculated from the difference in net hourly cation change in the presence and absence of ouabain. Sodium/potassium pump ratio was calculated by dividing sodium-active transport by potassium-active transport. Mean cell Na is the average RBC Na content during the period of incubation. Results are expressed as mean ± 1 SD of eight separate experiments.

DISCUSSION

Despite the same Na-K ATPase activity in term infant and normal adult RBC, it has been reported that active K transport is decreased in term neonatal RBC compared to normal adult erythrocytes (1). In these earlier studies, K transport was assessed utilizing ^{42}K, taking advantage of the fact that isotopic tracers allow precise determination of slow biochemical reactions which tax the sensitivity of chemical measurements. The utilization of tracer measurements, however, assumes that chemical behavior of the isotope is identical to the nonisotopic species. Unfortunately this assumption is not always correct. In the case of RBC monovalent cation transport, it has been demonstrated that measurements of ^{42}K influx, as well as ^{24}Na outflux, do not necessarily correlate with active cation transport since isotope-nonisotopic exchange reactions (^{24}Na-^{23}Na exchange and ^{42}K-^{41}K exchange) also occur across the red cell membrane (6). These exchange reactions might be acceptable if they represented a constant fraction of observed isotope movement, but, unfortunately, isotopic exchange varies significantly in different RBC and with the metabolic state of erythrocytes (6, 7).

In order to circumvent the problems associated with the use of radioisotopes, we designed experiments that would allow direct chemical measurement of Na and K. Active Na transport was measured by assessing changes in cell Na content over a period of time, while K transport was calculated on the basis of changes in medium K concentration during the same time interval. Moreover, these measurements were made under conditions where the transport rate was maximally stimulated following augmentation of the intracellular sodium concentration and stimulation of Na-K ATPase (2). The results of our study indicate that term neonatal RBC and normal adult RBC actively transport K at the same rate. Moreover, Na transport also is the same in term neonatal and normal adult RBC, and there is no difference in the Na:K pump ratio. These results are no surprise in view of the identical activity of Na-K ATPase in these cells, but they are different from a previous study which suggested that neonatal RBC have decreased active K transport (1). We have not determined the degree of isotopic exchange reactions in neonatal RBC, but considering the many biochemical differences between neonatal and adult erythrocytes (5), it is most likely that significant exchange differences exist between neonatal and adult RBC. For this reason, we believe the most meaningful data regarding the functional significance of Na-K ATPase can be obtained by chemical measurement of monovalent cation changes under conditions where the enzyme and cation transport rate are maximally stimulated.

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The Current Status of Auditory Brainstem Response Testing in Neonatal Populations

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Summary

The use of auditory brainstem response (ABR) for assessment of hearing in the neonate has not been without challenge. Although numerous articles have appeared, agreement regarding the utility of neonatal ABR testing does not exist. In review of the current studies and commentaries, a clear majority are favorable to neonatal ABR testing. These studies along with current test procedures are discussed.

Abbreviations

ABR, auditory brainstem response
NICU, neonatal intensive care unit
COG- Crib-O-Gram

Since the initial reporting of ABR in 1970 by Jewett *et al.* (26), the potential of accurate evaluation of auditory function in the neonate has existed. With the subsequent reporting of use in the NICU in 1975 by Schulman-Galambos and Galambos (38), procedures for the assessment of that potential were initiated. Since 1975, the research reported in the literature has grown exponentially each year. In spite of the large and increasing amount of research reported, severl issues have remained unresolved, *viz.*, the applicability, reliability, and validity of neonatal ABR testing. In fact, because of these and other factors, some authors have begun to seriously question using ABR with neonates (13, 35, 42).

The need for early identification of infants with hearing loss is apparent. Normal language, learning, and social skill acquisition is contingent upon hearing. Although the incidence of hearing impairment in the general neonatal population is relatively low (0.26%) (41), in NICU neonates the incidence is considerably higher (2–10%) (16, 39). Furthermore, the improved survival rate of neonates born weighing less than 1500 g (19, 31) has increased the pool of infants in which a high incidence of hearing impairment is seen (16).

Because the need for early identification of hearing loss does exist and the current procedures that could identify hearing impairment in the neonate are open to question (ABR), a critical review of ABR testing is in order. The purpose of this article is to summarize the current ABR literature, both pro and con, and to draw conclusions regarding its use based on the literature and the experience of the writer. The summary will not attempt to review early ABR literature primarily due to excellent reviews that are already published. The interested reader, however, would find Downs (13) very thorough.

The current literature pertinent to ABR testing of neonates and NICU populations is discussed and from several aspects (5–7, 11, 12, 17, 20, 25, 34, 36, 47). Weber (47) and Cox *et al.* (5, 6) have reported norms while Mjoen (32), Hecox *et al.* (20), Horning (24), and Cox *et al.* (7) have explored test reliability. The influence of various factors on the ABR have been explored by Barden and Peltzman (2), Bernard *et al.* (4), Galambos and Despland (15), Kileny *et al.* (27), Marshall *et al.* (29), Hecox and Cone (21), Hecox *et al.* (20), Cox *et al.* (8), and Roberts *et al.* (35). ABR as a screening tool has been reported by Horning (24), Crowell *et al.* (9), Hecox *et al.* (20), and Salamy *et al.* (37), while Simmons (43) and Galambos *et al.* (17) have compared ABR with Crib-O-Gram testing. These citations illustrate some of the current literature that is pertinent to neonatal ABR testing. In the following sections, specific aspects are discussed separately.

TEST PROTOCOL

The ABR protocol has undergone many refinements, a number of which have been facilitated by advances in commercial equipment. Factors considered in discussing current protocols include placement of the electrode, selection of intensity levels, test environment, state of the neonate, nature of the stimulus, and rate of presentation.

The most popular electrode configuration has usually been vertex (Cz) active with ipsilateral mastoid or earlobe reference and contralateral mastoid or earlobe as ground (2, 4, 9, 12, 20, 27, 34, 36). With possible fontanel problems, however, several authors have found forehead placement of the active electrode instead of the vertex to be effective and simple (7, 18, 25, 28–30, 32, 33, 35).

Intensity levels are typically designated as normal hearing level (as before a jury of normal listeners), hearing level (corresponds to manufacturer's dial reading), sound pressure level (0.0002 dyne/cm^2), sensation level (amount of intensity above individual threshold), and peak equivalent (equivalent amplitude of pure tone on oscilliscopic display). For simplicity sake, normal hearing level is best to use with periodic rechecks either behaviorally or with sound pressure level measure once normal hearing level has been determined.