RACIAL DIFFERENCES IN BUMETANIDE-SENSITIVE COTRANSPORT AND N-ETHYLMALEIMIDE-STIMULATED POTASSIUM EFFLUX

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Summary

Racial differences in erythrocyte potassium effluxes mediated
by two loop-diuretic sensitive modes of cotransport were
compared. In red cells loaded to contain approximately equimolar
amounts of sodium and potassium, black subjects had lower
bumetanide-sensitive sodium-dependent net potassium effluxes as
compared to whites. In fresh, washed erythrocytes pretreated with
N-ethylmaleimide (NEM), maximal net potassium efflux was greater in
blacks than in whites. NEM-stimulated potassium efflux was
partially inhibited by bumetanide but only at very high
concentrations. The quantitative differences in these two modes of
potassium efflux suggest that NEM-stimulated potassium efflux is
not an altered mode of sodium-dependent potassium efflux.

We (1) and others (2-6) have noted a striking racial difference in the
maximal rates of furosemide-sensitive effluxes of sodium and potassium from
sodium-loaded erythrocytes. In addition to a race-related alteration in the
activity of furosemide-sensitive transport, deviations from the expected 1 Na:1
K stoichiometry of classical sodium-potassium cotransport have been noted in
blacks (1,5,6). The description in sheep (7,8) and human (9-11) erythrocytes
of a ouabain-insensitive potassium efflux pathway, stimulated by the sulfhydryl
reagent N-ethylmaleimide (NEM), partially sensitive to furosemide and capable
of mediating net potassium efflux in the absence of sodium efflux, suggested a
possible explanation for the racial differences in furosemide-sensitive
fluxes. We therefore measured maximal net ouabain-insensitive sodium and
potassium effluxes in red cells loaded with equimolar amounts of the two ions
and also in fresh cells treated with NEM. We demonstrated that while
bumetanide-inhibited sodium and potassium transport is depressed in blacks,
NEM-stimulated potassium efflux is greater, suggesting a dissociation between
these two systems. It is possible that the NEM-stimulated pathway contributes
to previously observed racial differences in furosemide-sensitive cation
fluxes.

Methods

Eleven white and an equal number of black normal volunteers, with no known
cardiovascular, renal or endocrine disorders, were recruited by public adver-
tisement. Race was self-determined, and no attempt was made to control for
other ethnic derivation. Erythrocyte cation and water composition were meas-
ured in fresh washed cells. Maximal ouabain-insensitive sodium and potassium

effluxes were determined in cells loaded by a nystatin technique with equimolar amounts of sodium and potassium as previously described (1), with the exception that 0.1 mM bumetanide (Bumex®, Hoffman-LaRoche, Nutley, N.J.) was substituted for 1 mM furosemide when required. For determination of NEM-stimulated effluxes, red cells were washed three times with an ice-cold washing solution consisting of (mM) 75 $MgCl_2$, 85 sucrose, 10 TRIS-MOPS (pH 7.4 at 4° C), then suspended at an approximate hematocrit of 10% in a solution (MS) of (mM) 75 $MgCl_2$, 85 sucrose, 10 glucose, 0.1 ouabain, 10 TRIS-MOPS (pH 7.4 at 37° C) and transferred to a shaking water bath at 37° C. At zero time, NEM (Sigma Chemical Co., St. Louis, MO), freshly prepared as a 1 M stock in DMSO, was added to a final concentration of 1 mM. The cells were incubated in the presence of NEM for 15 minutes and were then removed and washed three times in MS. Aliquots of the washed cells were taken for determination of cell elec-trolytes and water, and packed cell hematocrit was determined in duplicate. The remaining cells were suspended at a hematocrit of 2-4% in MS, mixed and aliquoted into six tubes. These tubes were incubated in a shaking water bath at 37° C and duplicates were removed at 10, 20 and 30 minutes, iced for one minute and centrifuged at 2000 g for five minutes. The supernatants were removed and analyzed by atomic absorption spectrophotometry (Perkin-Elmer 2380 AA spectrophotometer, Perkin-Elmer Corporation, Norwalk, Connecticut) for sodium and potassium concentrations. Efflux rates were determined by linear regression fits of the external cation concentrations. Regression coefficients (r) of $\geq$ 0.98 were considered technically satisfactory. In the experiment to determine the response of NEM-stimulated potassium efflux to bumetanide, the drug was prepared as a 3 M solution in DMSO and an appropriate volume of DMSO was added to the control tube. Data were analyzed by t-test with pairing when appropriate and p values of $\leq$0.05 were considered significant.

Results

     Erythrocyte cation and water contents: Black subjects had a significantly higher cell sodium content in fresh, washed cells when compared to whites. Cell potassium was lower and cell water was slightly higher in blacks, but neither difference was significant.

TABLE I

Erythrocyte Electrolyte and Water Contents in Fresh, Washed Cells
(mean $\pm$ SD, n = 11 for both groups)

	Whites	Blacks
Sodium	8.07 $\pm$ 0.87	9.39 $\pm$ 1.50*
Potassium	97.77 $\pm$ 4.38	93.07 $\pm$ 4.74
Water	64.72 $\pm$ 1.30	65.95 $\pm$ 2.14

* p<0.05

     Bumetanide-sensitive effluxes from nystatin-treated, sodium-loaded cells: Bumetanide-sensitive sodium and potassium effluxes were significantly lower in blacks than in whites, as were the rate coefficients ($k_{Na,K}^{BSens.}$) for each ion.   Ouabain-insensitive, bumetanide-insensitive effluxes were not different, nor were the respective rate coefficients ($k_{Na,K}^{BInsens.}$).

TABLE II

Effect of Bumetanide on Ouabain-Insensitive Sodium and Potassium Effluxes
And Rate Coefficients (k) From Nystatin-Treated Erythrocytes
(mean ± SD, n = 11 for both groups)

	Whites	Blacks
Initial Cell Contents:		
Sodium (mmoles/l cells)	54.60 ± 5.66	60.38 ± 3.11*
Potassium (mmoles/l cells)	52.19 ± 2.31	52.98 ± 5.03
Water (%)	65.18 ± 1.75	66.09 ± 1.55
Bumetanide-sensitive Effluxes:		
Sodium (mmoles/l cells · h)	0.568 ± 0.222	0.192 ± 0.173 **
$k^{Na}_{BSens.}$ (h^{-1})	0.0107 ± 0.0046	0.0036 ± 0.0034**
Potassium (mmoles/l cells · h)	0.633 ± 0.148	0.200 ± 0.159**
$k^{K}_{BSens.}$ (h^{-1})	0.0121 ± 0.0029	0.0038 ± 0.0033**
Bumetanide-insensitive Effluxes:		
Sodium (mmoles/l cells · h)	1.433 ± 0.599	1.302 ± 0.424
$k^{Na}_{BInsens.}$ (h^{-1})	0.0264 ± 0.0116	0.0216 ± 0.0075
Potassium (mmoles/l cells · h)	1.053 ± 0.437	1.046 ± 0.268
$k^{K}_{BInsens.}$ (h^{-1})	0.0201 ± 0.0082	0.0200 ± 0.0068

* $p < 0.05$, ** $p < 0.01$, blacks versus whites.

Effects of NEM treatment: Blacks showed greater net potassium efflux compared to whites after exposure of cells to 1 mM NEM for 15 minutes. No sodium efflux was detectable in NEM-treated cells in either racial group. Exposure of cells to NEM in the presence of chloride resulted in a similar significant decrease in the percentage of cell water in both racial groups (whites: -2.49 ± 1.59, $p < 0.01$ versus fresh, washed cells by paired t-test; blacks: -2.60 ± 1.39, $p < 0.01$ versus fresh, washed cells by paired t-test). Such shrinkage can be prevented by the use of nitrate substitution for chloride (12). The slight increases in sodium and potassium content in washed cells following NEM treatment as compared to fresh cells (Table I) were not statistically significant.

TABLE III

Effect of NEM Treatment on Cell Contents, Net Potassium Efflux
and Potassium Efflux Rate Coefficient (k^{K}_{NEM})
(mean ± SD, n = 11 for both groups)

	Whites	Blacks
Initial Cell Contents:		
Sodium (mmoles/l cells)	8.01 ± 1.21	9.69 ± 2.06*
Potassium (mmoles/l cells)	98.58 ± 5.09	97.56 ± 7.65
Water (%)	62.24 ± 0.60	63.36 ± 1.37
K efflux (mmoles/l cells)	8.22 ± 2.39	12.51 ± 2.83**
k^{K}_{NEM} (h^{-1})	0.0831 ± 0.0253	0.1283 ± 0.0259**

* $p < 0.05$, ** $p < 0.01$, blacks versus whites

Bumetanide inhibited a substantial fraction of the NEM-stimulated potassium efflux, but very high concentrations were required. In the example shown, 50% inhibition of the NEM-stimulated potassium efflux was achieved at approximately 1.0 mM concentration of bumetanide, and complete inhibition was not obtained with the highest concentration tested, 3 mM, confirming the earlier findings of Lauf et al (11).
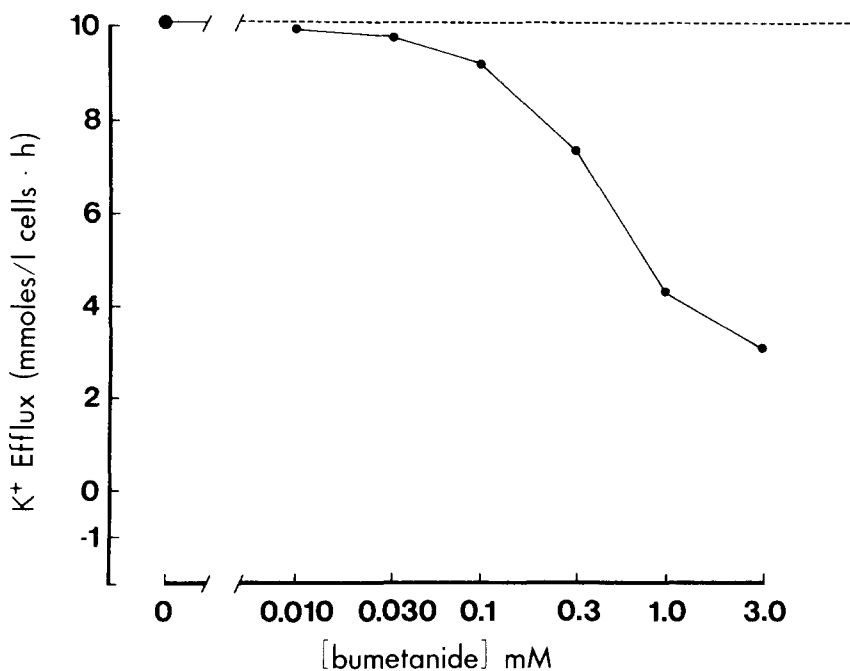

FIG. 1

Bumetanide inhibition of potassium efflux from erythrocytes exposed to NEM.   Dashed line represents level of potassium efflux in absence of bumetanide.   Approximately 70% inhibition was achieved at the highest concentration of bumetanide tested, 3 mM.

Discussion

Similarities in the effect of NEM on sheep and human cells, including a chloride dependence (8,10-12) and loop diuretic sensitivity of the induced potassium flux (10,12), suggest a similar mode of action in both species. While the precise details of the molecular site of the NEM effect are unknown, the suggestion was initially forwarded by Lauf et al that NEM-stimulated potassium efflux might represent an "uncoupled" action of the Na:K:2Cl cotransport system (9), although subsequent studies by the same authors have recently provided evidence suggesting that the potassium efflux unmasked by NEM is not an altered mode of Na:K:2 Cl cotransport (11). We studied the effect of NEM in blacks and whites because racial differences in furosemide-sensitive effluxes attributed to the classical Na:K:2Cl cotransport pathway have been well documented. The finding that NEM-stimulated sodium-independent potassium efflux is greater in blacks as compared to whites while, as expected, the maximal sodium-dependent bumetanide-sensitive potassium efflux is less, suggests that the two pathways are independent. We therefore favor the interpretation that NEM-stimulated potassium efflux represents a second loop-

diuretic sensitive pathway, perhaps related to K:Cl cotransport as suggested by Wiater and Dunham (10) and by Lauf et al (11). The physiological role of such a pathway in cation movements in normal human erythrocytes is not yet known.

Inhibition of NEM-stimulated potassium efflux by furosemide (10,12) and bumetanide (11) has been previously reported. This inhibition seems to be enhanced by the presence of external potassium or rubidium (and slightly by external sodium) (13). In the absence of external monovalent cations, the dose response curve to furosemide is shifted to the right by at least two orders of magnitude when compared to concentrations required for inhibition of the classical Na:K:2Cl cotransport system (9,11,13). We employed the loop diuretic bumetanide in this study because in our hands high concentrations of furosemide have occasionally resulted in hemolysis, especially in black subjects. Partial inhibition of NEM-induced potassium efflux was demonstrable at very high concentrations of bumetanide as has been reported by Lauf et al (11). Synergism between bumetanide and external cations has been noted in turkey red cells (15), and potassium may be important in modulating the inhibitory effect of bumetanide on NEM-stimulated potassium fluxes. Whether external ions alter the affinity of loop diuretics for the transport site involved in the NEM effect or modify the V_{max} of potassium transport is unknown, but Lauf has suggested a complex interaction based on his work with furosemide in sheep red cells (13). Further characterization of the racial difference in NEM-stimulated potassium efflux should include studies of the effect of bumetanide and the influence of external cations.

Racial differences, confirmed by this study, have previously been noted for red blood cell sodium content (16) and furosemide-sensitive sodium and potassium effluxes (1). The relationship of the present observation to these or other race-related membrane differences in sodium-lithium countertransport (1,4,5) and passive membrane permeability to lithium (1) is unknown, as this study represents the first report of an accelerated ouabain-insensitive potassium transport system in blacks. Additionally, the considerable interindividual variability for NEM-stimulated potassium efflux within each race suggests the need for studies of other factors determininig the activity of this system. Further work should clarify the role of the NEM-stimulated pathway in physiologic potassium movements in red blood cells and possibly in other tissues.

Acknowledgments

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