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FUROSEMIDE-SENSITIVE K^+ CHANNEL IN GLIOMA CELLS BUT NOT NEUROBLASTOMA CELLS IN CULTURE

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A furosemide-sensitive, ouabain-insensitive [$^{86}Rb^+$] uptake is described in glioma cells in culture which is dependent upon external Na^+, K^+, and Cl^- concentrations. This transport activity was also inhibited by bumetanide at 100-fold lower concentrations than furosemide. Furosemide-sensitive swelling of glioma cells is demonstrated and this activity is dependent upon external Na^+ and K^+ in a manner similar to [$^{86}Rb^+$] uptake. This transport activity was not detected in neuroblastoma cells and the possible relevance of these findings to extracellular K^+ buffering by glia is discussed.

Glial cells exhibit a selective permeability to K^+ and have been postulated to buffer extracellular K^+ concentration fluctuations during neuronal activity (1-4). Experimental evidence has led to the postulation that non-electrogenic K^+ fluxes might contribute to K^+ buffering by glia and might involve ions other than K^+, but the mechanism by which this occurs remains speculative (5-7). It has been shown that astrocytes have increased, and apparently saturable, K^+ uptake in the presence of increased external K^+ concentrations part of which is ouabain-insensitive (6,7). In this paper, we describe a ouabain-insensitive [$^{86}Rb^+$] uptake in glioma but not neuroblastoma cells which is sensitive to both furosemide and bumetanide, which is external Na^+, K^+, and Cl^- dependent, and is very similar to the Na^+ plus K^+ cotransport systems described in Ehrlich ascites tumor cells (8,9) and avian erythrocytes (10,11). This activity is qualitatively and quantitatively consistent with experimental observations showing ouabain-insensitive K^+ flux in glial cells *in vitro* and *in vivo* (5-7, 12,13).

Materials and Methods

The clonal cell lines used in this study and cell culture conditions are extensively described elsewhere (14,15). The C6BU-1 glioma line is a bromodeoxyuridine-resistant mutant of the rat C6 glioma and the N18TG2 neuroblastoma line is a 6-thioguanine-resistant mutant of the N18 mouse neuroblastoma. Cells were grown for at least 72 hrs in Dulbecco's Modified Eagles' medium supplemented with 10% fetal calf serum before use. Assays were routinely performed on cells plated in 12-well (22mm^2/well) plastic dishes.

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Transport assays were performed in a buffer consisting of 10mM Hepes adjusted to pH 7.35 with Tris, 80mM NaCl, 2.8mM KCl, 2.3mM $CaCl_2$, 2.3mM $MgCl_2$, 21 mM $NaHCO_3$, 1mM Na_2HPO_4, 80mM choline chloride and 10mM glucose. Inhibitors were prepared as 500-fold concentrated stock solutions in dimethylsulfoxide. Controls were always performed with and without dimethylsulfoxide to test for any possible effects of the solvent and none were observed. Following a 15 min preincubation at 37°C, [^{86}Rb]Cl was added to a final specific activity of 100cpm/nmol K^+ in the buffer. Uptake was allowed to proceed for 15 min at 37°C and was terminated by aspirating the medium with isotope and washing the cells twice in ice-cold buffer. Zero times were performed by deleting the 15 min incubation at 37°C with the isotope. Cells were lysed by the addition of buffer containing 1% deoxycholate and 12μg DNase/ml. A 50μl aliquot was analyzed for [^{86}Rb] content by liquid scintillation counting. Protein was determined by the method of Lowry, et al (16) using bovine serum albumin in buffer containing deoxycholate and DNase as the standard. All data points represent the means of duplicate determinations from a minimum of three independent experiments.

For experiments where KCl concentrations were varied, reciprocal adjustments in the choline chloride concentrations were made to maintain isotonicity and NaCl concentrations were held constant at 80mM. For experiments where NaCl was varied, reciprocal adjustments in the choline chloride concentrations were made to maintain isotonicity and KCl concentrations were held constant at 2.8 mM. For experiments where Cl^- was varied, Na^+ and K^+ were added as the indicated anionic salt, choline was omitted from the medium and replaced by Na, and adjustments were made by reciprocally varying NaCl and the Na-salt concentration of the appropriate anion. Water uptake measurements were made using the [^{3}H]H_2O and [^{14}C] inulin method of Rottenberg, et al (17).

Results

Figure 1 shows the effects of varying external Na^+ and K^+ concentrations on the uptake of [$^{86}Rb^+$] into C6BU-1 glioma cells and N18TG2 neuroblastoma cells in the presence and absence of furosemide and ouabain. Although ouabain inhibition of [$^{86}Rb^+$] uptake is virtually complete in N18TG2 neuroblastoma cells (Fig. 1B and 1D), only 40% inhibition of [$^{86}Rb^+$] uptake by ouabain was observed in C6BU-1 glioma cells (Fig. 1A and 1C). The remaining [$^{86}Rb^+$] uptake observed in C6BU-1 glioma cells was found to be furosemide-sensitive as judged by a 60% inhibition of [$^{86}Rb^+$] uptake in the presence of furosemide and complete inhibition of [$^{86}Rb^+$] uptake when C6BU-1 glioma cells were treated with furosemide together with ouabain (Fig.1A and 1C). These data indicate that, under the experimental conditions employed, [$^{86}Rb^+$] uptake in N18TG2 neuroblastoma proceeds almost exclusively via the Na^+-K^+ ATPase whereas both the Na^+-K^+ ATPase and a diuretic-sensitive transport system are present in C6BU-1 glioma cells. Although the similarities between K^+ transport properties of rat C6 cells and normal astrocytes have been emphasized previously (18), it should be noted that data for [$^{86}Rb^+$] uptake in control and ouabain-treated C6BU-1 glioma presented in Fig. 1A and 1C are virtually identical to data published for primary cultures of rat astrocytes (7) in the presence of varying external K^+ concentrations.

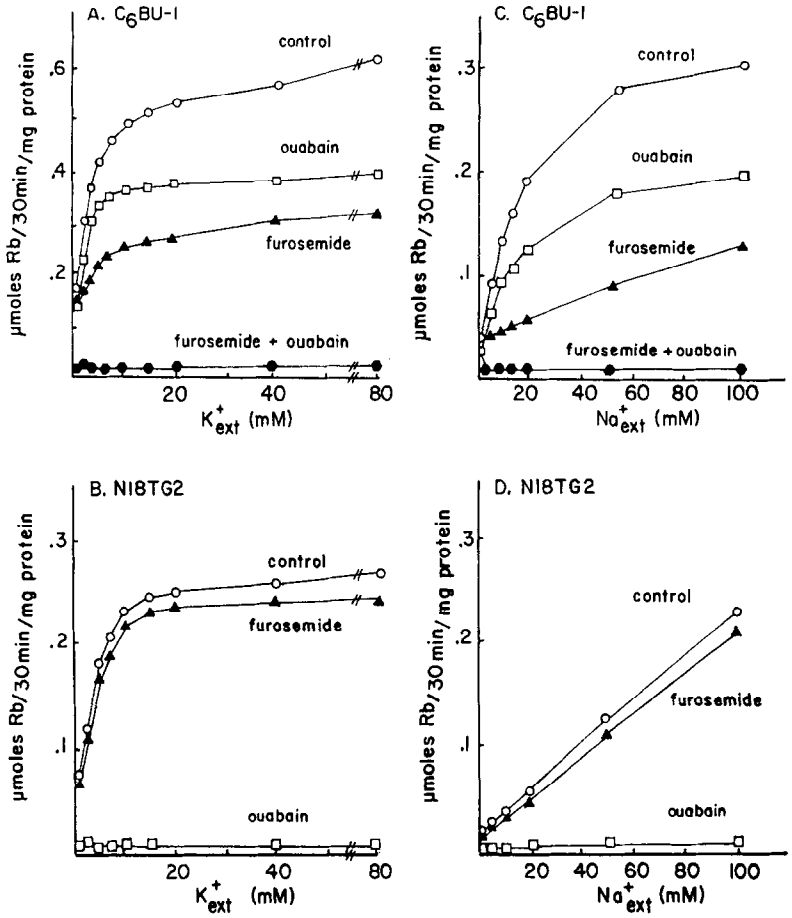

Figure 1.
Dependence of $[^{86}Rb^+]$ uptake on external K^+ (A and B) and Na^+ (C and D) concentrations in C6BU-1 glioma cells (A and C) and N18TG2 neuroblastoma cells (B and D). Cells were treated with ouabain (1mM), furosemide (500μM) and ouabain (1mM) together with furosemide (500μM). Assays of $[^{86}Rb^+]$ uptake are described in Materials and Methods.

Data in Fig. 2 show that complete replacement of external Cl^- with gluconate, acetate or thiocyanate resulted in $[^{86}Rb^+]$ transport that was entirely ouabain-sensitive in C6BU-1 glioma cells. Although these anions were shown to be incapable of substituting for external Cl^- in this system, Fig. 2 whows that Br^- appears to weakly substitute for Cl^-. Direct coupling of Cl^- fluxes to bumetanide-sensitive Na^+ plus K^+ cotransport in Ehrlich ascites tumor cells (8,9) and an external Cl^--dependence of furosemide-sensitive Na^+ plus K^+ cotransport in avian erythrocytes (10,11) have been reported. In both systems (9,11), Br^- was partially capable of substituting for Cl^-. Thus, it appears that the C6BU-1 glioma cells transporter is very similar, in terms of external ionic requirements, to the furosemide-sensitive transport process in these other cells.

Furosemide has been documented to be an inhibitor of several transport processes (10); while a second 5-sulfamoylbenzoic acid derivative, bumetanide,

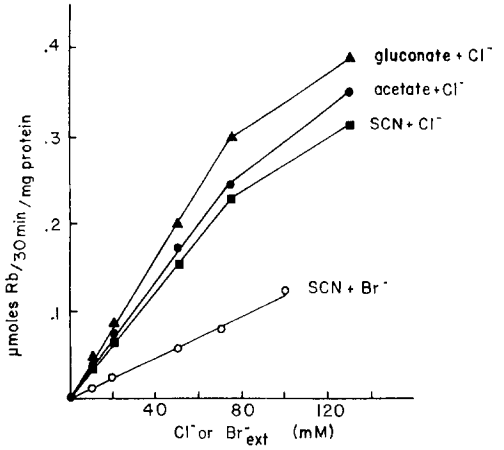

Figure 2.
Dependence of ouabain-insensitive $[^{86}Rb^+]$ uptake on external Cl^- concentrations in C6BU-1 glioma cells. Cells were treated with ouabain (1mM) or ouabain together with furosemide (500μM) and assayed as described in Materials and Methods.

has been shown to inhibit Na^+ plus K^+ cotransport at 100-fold lower concentrations than furosemide without interfering with processes such as anion self-exchange and the Na^+-K^+ ATPase (10). Data in Fig. 3 show that bumetanide was found to be 100-fold more effective than furosemide against ouabain-insensitive $[^{86}Rb^+]$ uptake in C6BU-1 glioma cells. In these experiments, neither furosemide nor bumetanide was observed to inhibit ouabain-sensitive $[^{86}Rb^+]$ pumping more than 10% at concentrations less than 1mM in both C6BU-1 glioma cells and N18TG2 neuroblastoma cells (not shown).

Figure 4 shows that furosemide-sensitive swelling of C6BU-1 glioma cells was found to be similar to furosemide-sensitive $[^{86}Rb^+]$ uptake in terms of

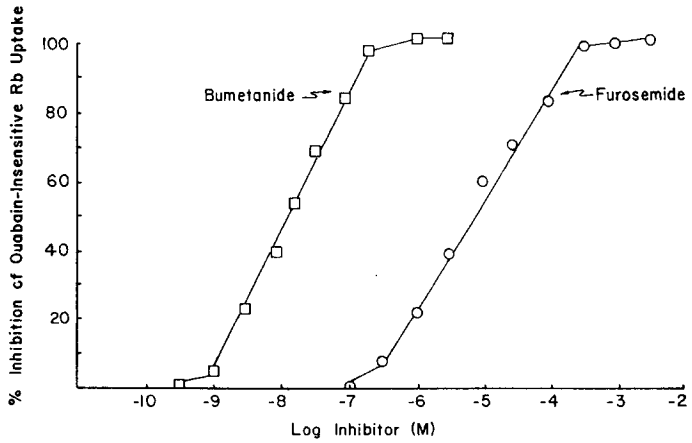

Figure 3.
Furosemide and bumetanide sensitivity of ouabain-insensitive $[^{86}Rb^+]$ uptake in C6BU-1 glioma cells. Cells were treated with the indicated concentrations of furosemide or bumetanide together with 1mM ouabain and assayed as described in Materials and Methods.

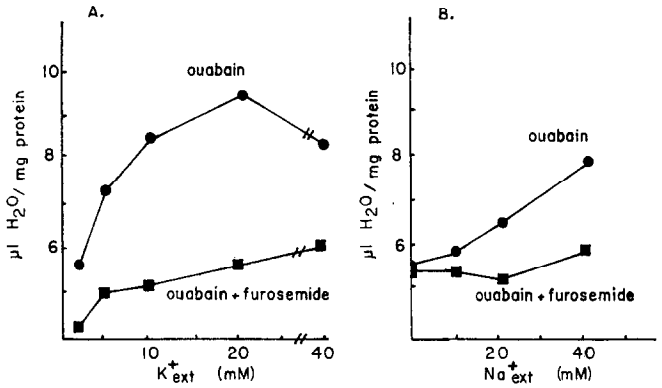

Figure 4.
External (A)K^+ and (B)Na^+-dependence of furosemide-sensitive water uptake in C6BU-1 glioma cells. Cells were treated with 1mM ouabain and 1mM ouabain together with 500µM furosemide and water uptake was determined as described previously (17).

dependence upon external K^+ and Na^+ concentrations (compare Fig. 1A with Fig. 4A and Fig. 1C with Fig. 4B). It is of more than casual interest that correlations between extracellular K^+ concentrations and glial swelling have been documented both in brain slices and primary astrocyte cultures (5-7,12,13) while the furosemide and bumetanide-sensitive Na^+ plus K^+ cotransport systems of Ehrlich ascites tumor cells and avian erythrocytes have been postulated to have cell-volume regulatory functions (8-10). Further, ethacrynic acid (a compound with diuretic properties similar to those of furosemide and bumetanide) has been shown to inhibit swelling in cerebrocortical slices from cat brain which was HCO_3-stimulated, was dependent upon external K^+ and involved the net transport of Na^+ and Cl^- (19).

Discussion

Data presented here provide evidence that a K^+ transport system is operative in C6BU-1 glioma cells which is sensitive to furosemide and bumetanide, which accounts for ouabain-insensitive [$^{86}Rb^+$]uptake, which is dependent upon external Na^+, K^+, and Cl^- concentrations, and which appears to be coupled to H_2O movement. This system is very similar to the ouabain-insensitive, furosemide and bumetanide-sensitive Na^+:K^+:Cl^-(1:1:2)-coupled transport system shown in Ehrlich ascites tumor cells (8,9) and suggested in avian erythrocytes (10,11). We suggest that a similar transport process may be operative in C6BU-1 glioma cells but not N18TG2 neuroblastoma cells in culture.

Because data for [$^{86}Rb^+$] uptake in primary astrocyte cultures in the presence and absence of ouabain (7) are quantitatively very similar to those reported here, we further suggest that a furosemide and bumetanide-sensitive K^+ channel may be present in normal astrocytes. This channel activity is

consistent with experimentally suggested non-electrogenic K^+ fluxes in glia and could conceivably play an important role in glial maintenance of K^+ homeostasis and osmotic regulation in brain (5-7,12,13).

Acknowledgements

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