DIVERSE EFFECTS OF FLUORIDE ON NA^+ AND K^+ TRANSPORT ACROSS THE RAT ERYTHROCYTE MEMBRANE

Natalia I Agalakova,[a] Gennadii P Gusev[a]

Sankt-Petersburg, Russia

SUMMARY: The cellular effects of fluoride (as F^- from NaF) on Na^+ and K^+ transport across the rat erythrocyte membrane were investigated using ^{22}Na as a tracer and nonradioactive Rb^+ as a K^+ congener. Exposure of rat erythrocytes to 20 mM NaF for 30–120 min considerably diminished (by 68%) Rb^+ influx into the cells, comparable to the inhibitory action of 1 mM ouabain. F induced a modest accumulation of cellular Na^+ and a loss of cellular K^+ significantly greater than the effects produced by ouabain. Removal of Ca^{2+} from the standard medium resulted in a significant decrease of F-induced Na^+ accumulation and ^{22}Na uptake and almost completely suppressed F-induced loss of K^+. Treatment with 1 mM amiloride and 0.2 mM bumetanide inhibited the F-induced Na^+ influx by 70% and 20%, respectively, suggesting an involvement of amiloride-sensitive pathways (Na^+/H^+ exchanger or Na^+ channels) and Na-K-2Cl^- cotransport. Addition of 50 µM Al^{3+} (activator of G-proteins) into incubation medium did not affect the F-induced alterations of both Na^+ influx and intracellular Na^+ and K^+ content. Treatment of the cells with 1 mM quinine or 1 mM Co^{2+} (blockers of Ca^{2+} transport), 1 mM amiloride, or 5 µM KN-93 (inhibitor of Ca^{2+}/calmodulin-dependent protein kinases) produced substantial inhibition of F-induced Na^+ accumulation by 40–48% and K^+ loss by 16–30%. These findings suggest that the observed changes in activity of a number of enzymes and intracellular Ca^+ concentration may mediate many of the early events involved in the response of rat erythrocytes to inorganic F.

Keywords: Erythrocyte membrane; Fluoride and ion transport; Intracellular Ca^{2+} concentration; K^+ and Na^+ transport; Protein phosphatases; Rat erythrocytes.

INTRODUCTION

Fluoride ion (F) is known for its exceptional biological activity and diverse effects on the living organisms. The ability of F to alter the activity of many cell enzymes and to affect various metabolic pathways, thereby causing cytotoxic effects, is well established.[1-4] F is also implicated in the transport of inorganic cations across the cell plasma membrane due to both the direct blockade of transport ATPases[5-7] and/or the inhibition of activity of protein phosphatases.[8-12] Many of the cellular effects of F can be attributed to its ability to form fluoroaluminates AlF_{3-5} that can activate G proteins,[13,14] which, in turn, results in modification of the cellular concentrations of the secondary messengers involved in the regulation of ion transport across the plasma membrane. In a number of cell types, F has been shown to enhance an intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$)[15-18] that might have a substantial influence on the membrane transport of Na^+ and K^+.

Red blood cells (erythrocytes) are convenient for elucidation of the cellular mechanisms underlying F action. It is well documented that exposure of human erythrocytes to F results in decrease of the cell ATP concentration and active

[a]For Correspondence: Dr NI Agalakova, PhD, [a]Laboratory of Comparative Physiology of Inorganic Ions, Sechenov Institute of Evolutionary Physiology and Biochemistry, 44 Moris Thorez Pr., Sankt-Petersburg, 194223, Russia; E-mail: agalak@iephb.ru.

extrusion of Na^+ as well as considerable loss of cellular K^+.[19-21] In the studies on red blood cells of several other species, including rats, F has been used as an activator of G-proteins and as inhibitor of protein phosphatases.[9,10,12,22-24] The aim of the present work was to conduct a detailed investigation of the influence of F on Na^+ and K^+ transport across the rat erythrocyte membrane and intracellular homeostasis of monovalent cations.

MATERIALS AND METHODS

Experimental animals: Eight-to-twelve-week-old male Wistar rats weighting 150–200 g were used for the experiments. Rats were housed in a temperature-controlled room at 20–23ºC under a 12-hr light/dark cycle with free access to the standard rat chow and tap water nominally free of F. All the experimental procedures were performed in accordance with the Russian Federation laws relating to experiments on laboratory animals. Seven animals were used for each series of experiments.

At the end the study periods, the rats were anesthetized with a lethal dose of ether, and blood from the carotid artery was collected into heparinized tubes containing cold washing solution of the following composition: 140 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM Tris-HCl (pH 7.4 at 5ºC). The blood was immediately centrifuged (3000 g at 5ºC for 5 min), plasma and the upper cell layer were discarded, and the erythrocytes were washed three times with the same solution. The resulting erythrocytes were re-suspended in a standard incubation medium to final hematocrit of 20–30%. The standard incubation medium contained 140 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Tris-HCl, and 10 mM glucose (pH 7.4 at 37ºC). Ca^{2+}-free medium contained all listed salts except $CaCl_2$ and was added with 0.1 mM EDTA-Na_2.

Rb^+ influx: All determination experiments were conducted at 37ºC. The erythrocytes were incubated in the standard media containing 4 mM RbCl in place of 4 mM KCl. Next, 20 mM NaF or 1 mM ouabain (final concentrations), singly or in combination, was added to the incubation media. The samples of the cell suspension were aspirated after 30, 60, or 120 min of incubation for measurement of intracellular Na^+, K^+, and Rb^+.

Cell ion composition: Red blood cells (RBCs) were washed with cold solution containing 110 mM $MgCl_2$ and 10 mM Tris-HCl (pH 7.4 at 4ºC), then lysed in distilled water. Na^+ and K^+ concentrations in the cell lysates were measured with a Flapho-40 flame photometer (Carl Zeiss, Germany). Rb^+ concentration was determined using SHIMADZU Atomic Absorbance Spectrophotometer AA-6800.

^{22}Na uptake: Unidirectional Na^+ influxes were measured using ^{22}Na as a tracer for 60 min when the isotope influx was linear as shown previously.[32] Aliquots (100 µL) of the cell suspension were added to the tubes (final hematocrit of 2–3%) containing 1 mL of the standard incubation medium and testing reagents. Then ^{22}Na was added to the test tubes (final activity ~3 µCi/mL) and its uptake for 60 min was determined. After incubation, 1 mL of the cell suspension was injected into 10 mL of cold $MgCl_2$-Tris-HCl solution. Aliquots of supernatant were taken

to determine radioactivity of the medium. The erythrocytes were washed twice with the same solution, lysed with 5% trichloracetic acid, and aliquots of the lysates were taken for counting. Radioactivity of incubation medium and cell lysates was measured using a beta-counter 1209 RACKBETA ("LKB", Wallac). Na^+ influx was calculated as

$$J_{Na} = \frac{A_{RBC} \cdot C_{Na}}{A_m \cdot t}$$

where A_{RBC} and A_m are the radioactivity of 1 mL cells and 1 mL medium, respectively, C_{Na} is the Na^+ medium concentration (OM/mL), and t is the incubation time (hr).

Chemicals: All reagents, buffers, and salts were analytical grade and purchased from Sigma (St. Louis, MO, USA) or MP Biomedicals (Irvine, CA, USA). Stock solutions of amiloride, bumetanide, and ouabain were dissolved in DMSO (dimethylsulfoxide). The same volumes of DMSO were added to the control samples. Stock solution of NaF (500 OM) was prepared on distilled water. ^{22}Na was obtained from ISOTOP (Sankt-Petersburg, Russia).

Statistical analysis: All data were processed by SigmaPlot software package version 6.0 (Jandel Scientific). Statistical differences of the measured variables were assessed using Student's t test for paired data. Results are mean values with standard errors (± SE). P values less than 0.05 were considered significant.

RESULTS

Effect of F on Rb^+ influx into rat erythrocytes: Since F is known to influence the active transport of monovalent cations across cell membranes, its effect has been compared to that of ouabain, a selective inhibitor of Na^+-K^+-ATPase. Figure 1 shows that the intracellular accumulation of Rb^+ as a substitute for K^+ is considerably diminished in the presence of ouabain or F as compared to control value. The inhibitory effect of F was found to be somewhat lower, yet statistically significant, compared to that of ouabain. Rb^+ influx in the presence of a combination of these agents did not differ from that in the presence of ouabain alone (1.46 ± 0.11 vs 1.45 ± 0.08 mM/L/hr).

Effect of F on cellular Na^+ and K^+ content in rat erythrocytes: Intracellular concentrations of Na^+ increased in the presence of both ouabain and F as compared to control (Figure 2), reaching statistically significant values 60 and 120 min after incubation. F produced substantially greater alterations in the Na^+ content as compared to ouabain. On the other hand, intracellular K^+ concentration diminished during incubation of the rat erythrocytes with ouabain or F (Figure 3), although a statistically significant decrease of K^+ concentration was observed only after 120 min of incubation with ouabain. An addition of F induced a more pronounced effect on the cellular K^+ content as compared to ouabain, with K^+ concentration being gradually decreased in the presence of F within 60 and 120 min. As can be seen from Figures 2 and 3, treatment of the rat erythrocytes with combination of F and ouabain did not cause any additional effect on the content of intracellular cations as compared to F alone. Intracellular concentrations of

monovalent cations in the control cells remained unchanged over the entire period of incubation.

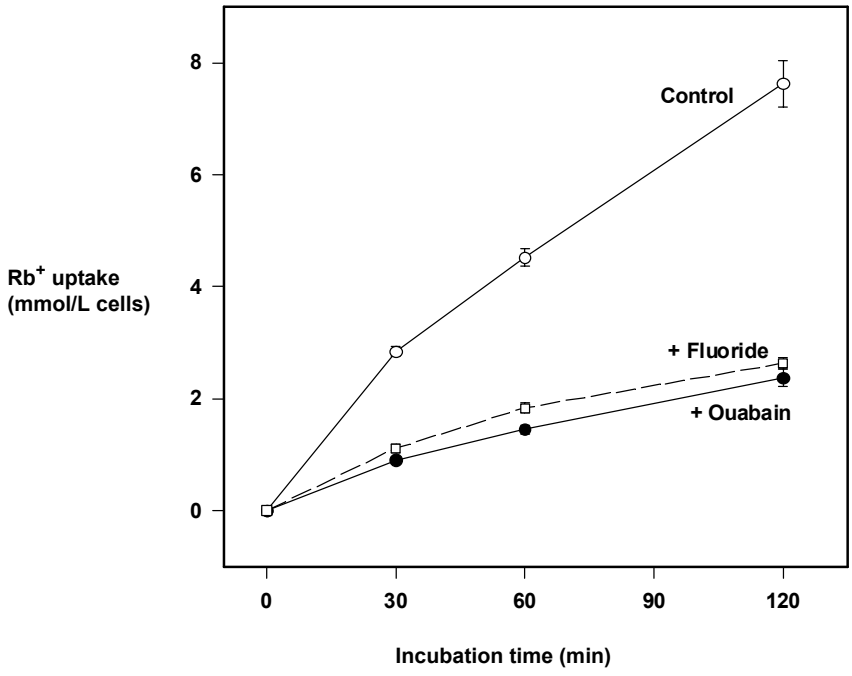

Figure 1. Effect of F on Rb^+ influx into rat erythrocytes. Intracellular Rb^+ content was determined as described in Materials and Methods. Values are means ± SE for 7 experiments.

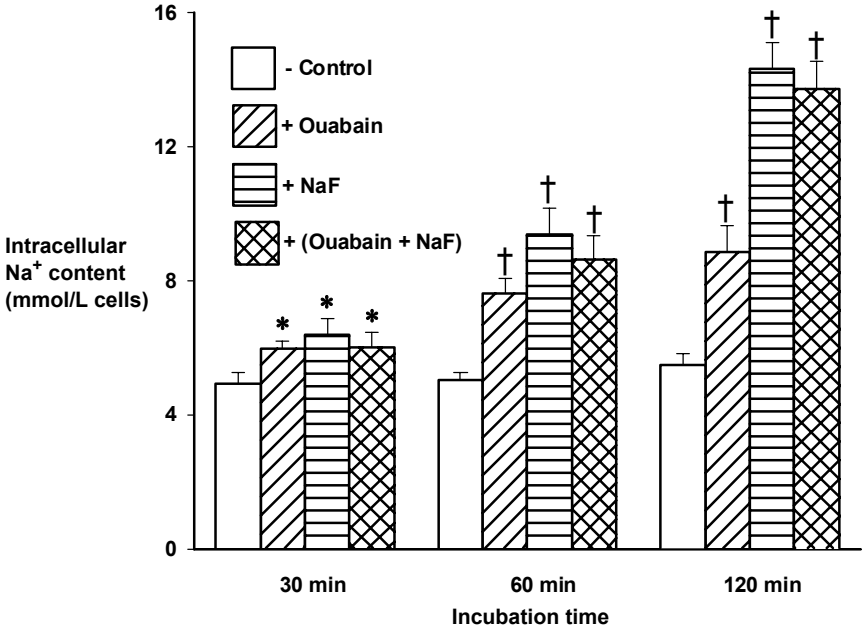

Figure 2. Effect of F on the cellular Na^+ content. Values are means ± SE for 7 experiments. *$p<0.01$, †$p<0.001$ as compared to controls.

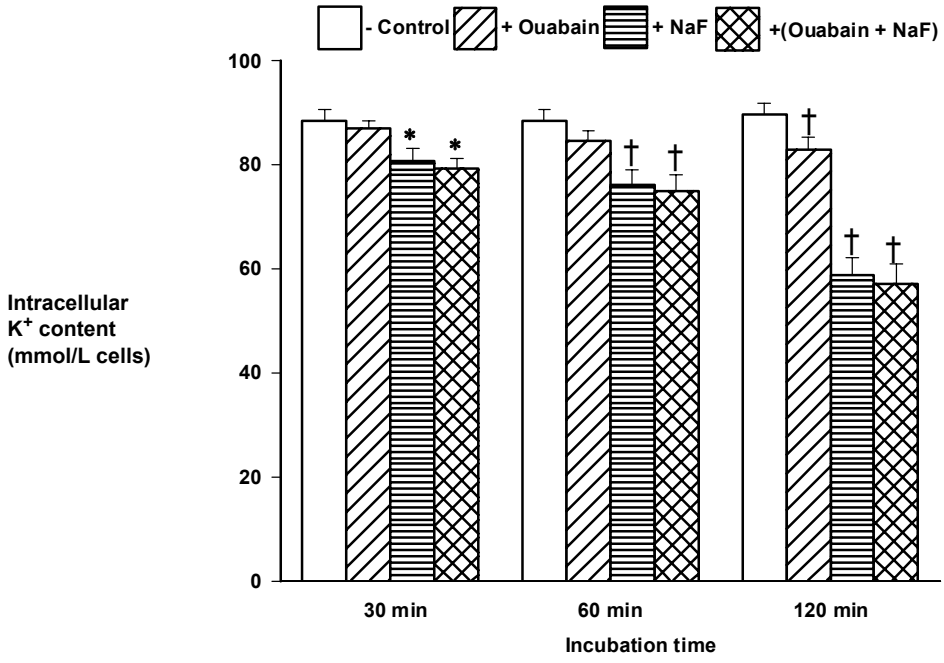

Figure 3. Effect of F on intracellular K^+ concentration. Values are means ± SE for 7 experiments. *p<0.01, †p<0.001 vs controls.

Role of Ca^{2+} in the effects of F on intracellular Na^+ and K^+ contents: The exposure of rat erythrocytes to F or ouabain in a Ca^{2+}-free medium for 2 hr also resulted in statistically significant accumulation of Na^+ and loss of K^+ (Table). Accumulation of Na^+ in the presence of ouabain was substantially lower than the changes in Na^+ content induced by F or combinations of F and ouabain. However, both agents individually or in their combination induced small identical losses of K^+ from the rat erythrocytes.

Table. Effects of F and ouabain on the content of Na^+ and K^+ (mM/L) in rat erythrocytes incubated in Ca^{2+}-free medium for 2 hr

Cation	Control	Ouabain 1 mM	Fluoride 20 mM	Ouabain + Fluoride
Sodium	6.31 ± 0.18	10.6 ± 0.33*	11.8 ± 0.26*	11.6 ± 0.46*
Potassium	95.3 ± 0.9	91.4 ± 1.1*	90.7 ± 2.7*	87.7 ± 3.8*

Average values ± SE for 7 experiments are presented. *p<0.01 as compared to control.

In the next series of experiments, the content of monovalent cations was studied in the cells exposed to F for 60 and 120 min in the standard and Ca^{2+}-free media simultaneously. After removal of Ca^{2+} from the incubation medium, F-induced accumulation of Na^+ in erythrocytes was three times less than that observed in the standard medium (Figure 4). Intracellular K^+ concentration did not vary between the control and F-treated cells during 60 min of incubation in a Ca^{2+}-free medium

(Figure 5), but statistically significant decrease of erythrocyte K^+ content was observed after 120 min. In the standard medium, however, an exposure of the cells to F caused a substantial loss of intracellular K^+. The removal of Ca^{2+} from the medium in the absence of F did not influence the erythrocyte Na^+ and K^+ contents during 2 hr of incubation.

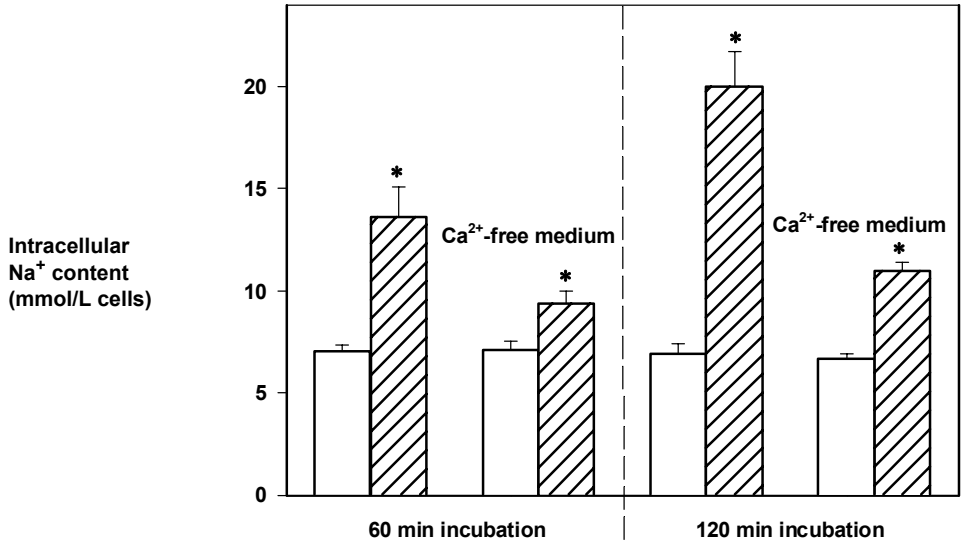

Figure 4. Effect of F on the content of Na^+ in rat erythrocytes incubated in the standard and Ca2+-free media. White columns = controls; hatched columns = 20 mM F. Values are means ± SE for 7 experiments. *p<0.001 vs controls.

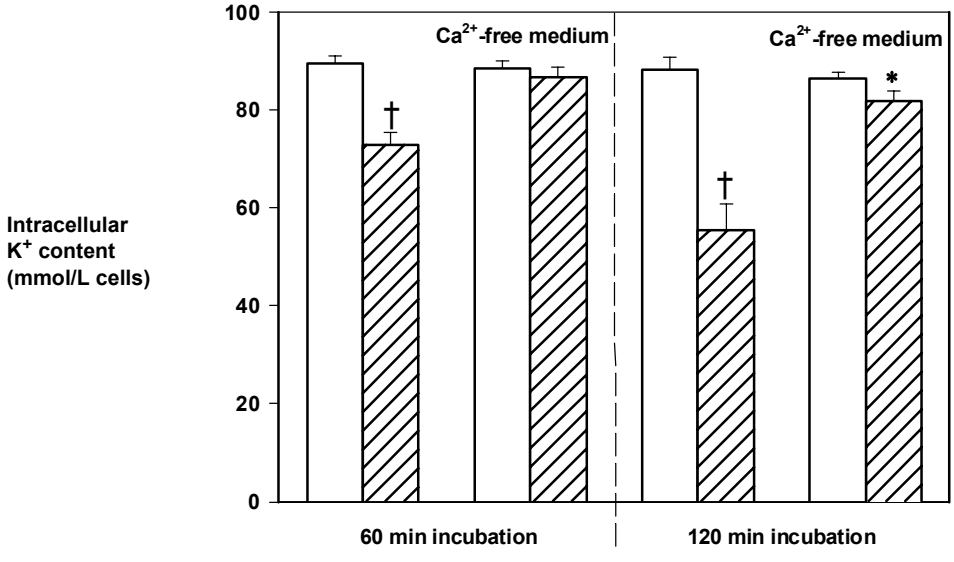

Figure 5. Effect of F on K^+ concentration in the rat erythrocytes incubated in the standard and Ca^{2+}-free media. White columns = controls; hatched columns = 20 mM F. Values are means ± SE for 7 experiments. *p<0.02, †p<0.002 vs controls.

As part of further tests, the rat erythrocytes were treated with F in combination with the inhibitors of various ion transport systems (quinine, Co^{2+}, amiloride), an inhibitor of Ca^{2+}/calmodulin-dependent kinase KN-93, or with Al^{3+} (activator of G-proteins) (Figure 6). The changes in intracellular Na^+ and K^+ concentrations upon combined addition of F and Al^{3+} did not differ from those induced by F. A 40–50% decrease in Na^+ accumulation was observed in F-treated cells in the presence of quinine, Co^{2+}, amiloride, or KN-93. These substances also diminished the cellular F-induced K^+ loss, but to a lesser extent (16–30%) than Na^+ accumulation.

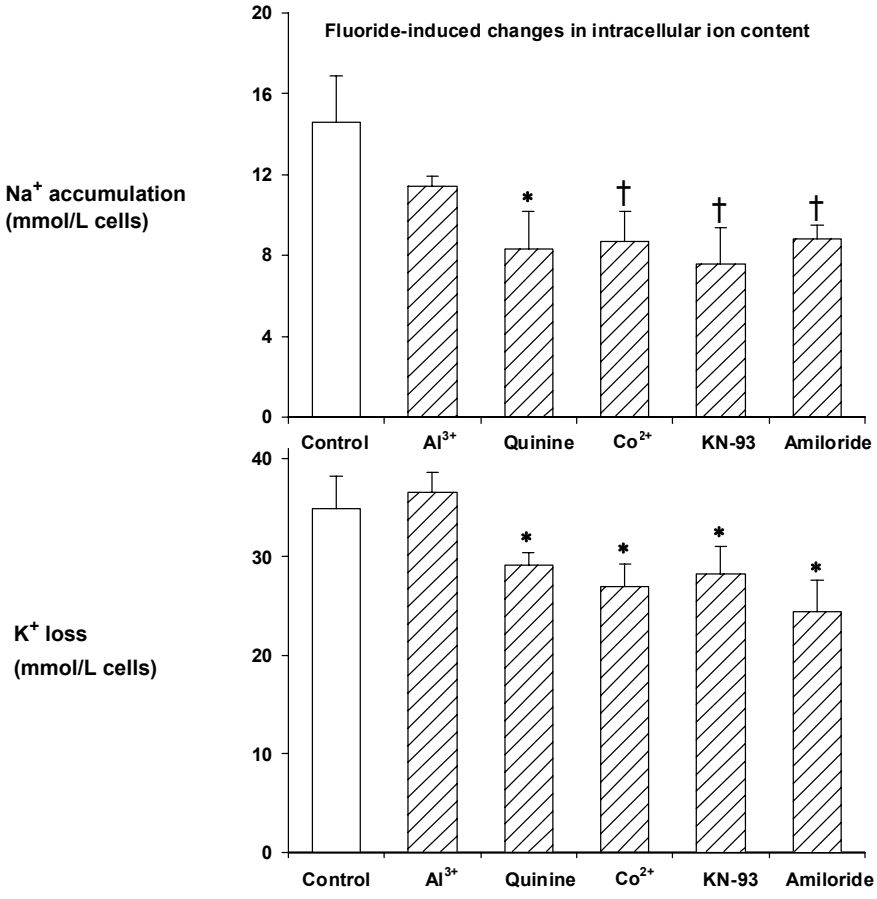

Figure 6. Effect of Al^{3+} and Co^{2+} ions, quinine, amiloride, and KN-93 on the F-induced accumulation of Na^+ and loss of K^+. Rat RBCs were incubated for 2 hr in the standard medium in the absence and in the presence of 20 mM NaF alone or in combination with 50 μM $AlCl_3$, 1 mM $CoCl_2$, 1 mM quinine, 1 mM amiloride, 5 μM KN-93. Values are means ± SE for 7 experiments. *$p<0.05$, †$p<0.02$ as compared to controls.

Effect of F on Na^+ influx in the rat erythrocytes: Exposure of the cells to F resulted in significant activation of ^{22}Na uptake by erythrocytes incubated in both standard and Ca^{2+}-free media (Figure 7). In a Ca^{2+}-free medium, however, F-induced Na^+ influx was substantially diminished as compared to that in normal medium ($p < 0.001$). The removal of Ca^{2+} from the control medium had no

influence on Na^+ influx into rat erythrocytes. Preincubation of the cells for 60 min did not cause any effect on the Na^+ influx in erythrocytes. On the other hand, the stimulatory effect of F was substantially intensified after 60 min preincubation of the cells in standard medium.

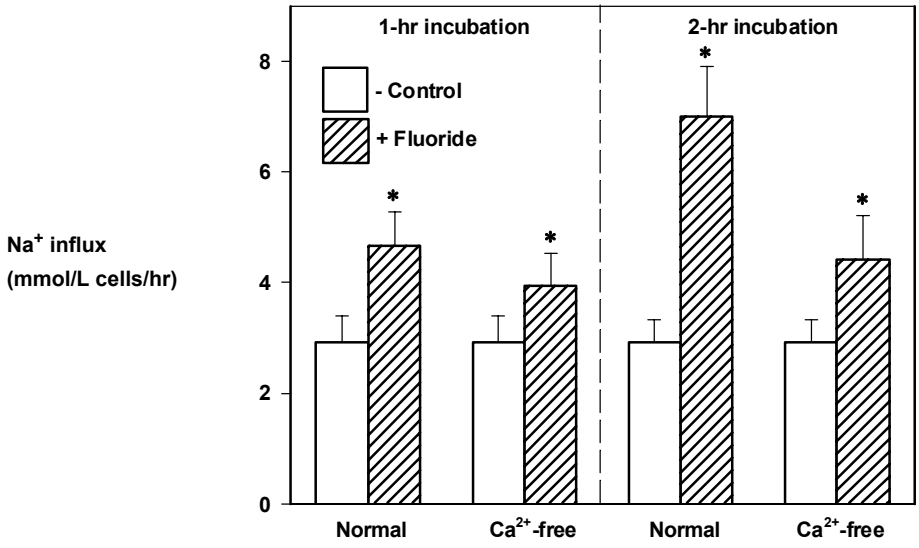

Figure 7. Effect of F on ^{22}Na uptake by the rat erythrocytes incubated in parallel in the standard and Ca^{2+}-free media. ^{22}Na was added at zero time (1-hr incubation) or after 1-hr preincubation with 20 mM F (2-hr incubation) and its uptake for 1 hr was determined as described in Materials and methods. Values are means ± SE for 7 experiments. *p<0.001 as compared to controls.

An addition of the G-proteins activator Al^{3+} to the incubation medium did not affect the Na^+ influx in erythrocytes (Figure 8).

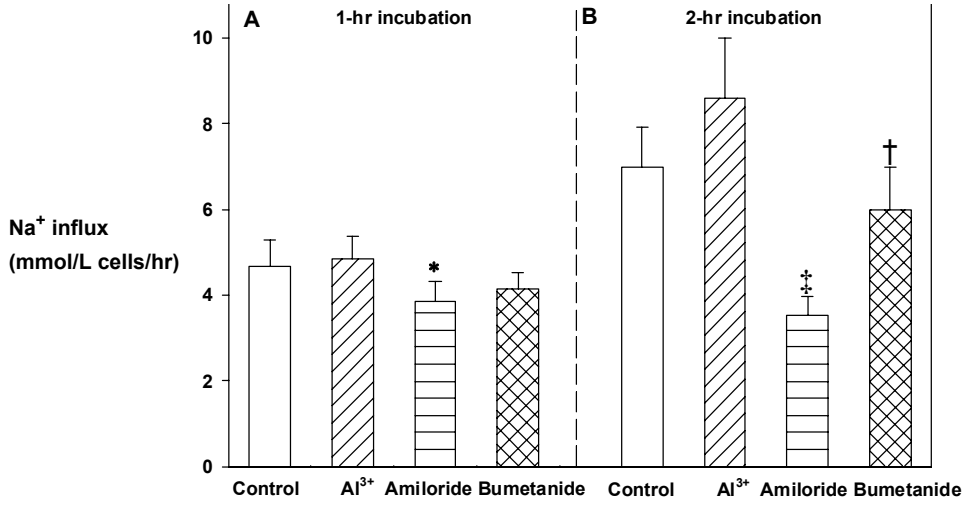

Figure 8. Effect of G-proteins activator (Al^{3+}) and Na^+ transport inhibitors (amiloride, bumetanide) on the F-induced Na^{22} uptake. Rat RBCs were incubated in the standard medium containing 20 mM NaF solely or in combination with 50 μM Al^{3+}, 1 mM amiloride, or 0.2 mM bumetanide. ^{22}Na uptake for 1 hr was determined without (1-hr incubation) or with 1-hr preincubation (2-hr incubation). Values are means ± SE for 7 experiments. *p<0.01, †p<0.02, ‡p<0.001 as compared to controls.

Treatment of the cells with amiloride for 60 and 120 min produced a statistically significant decrease of the F-induced Na^+ influx. In the presence of bumetanide, a significant decrease of Na^+ influx in the cells was observed only after 120 min treatment

DISCUSSION

The results of this study illustrate the diverse and complex effects F has on Na^+ and K^+ transport across the rat erythrocyte membrane. As seen here, F exerts a pronounced inhibitory impact on the Na^+-K^+-pump activity similar to that of ouabain (Figure 1). One of the possible mechanisms of F action is a suppression of energy exchange via well-known inhibition of glycolysis—the main energy source for active ions transport in mammalian erythrocytes. The ability of F to decrease the cell ATP concentration and ^{22}Na efflux has been demonstrated for human erythrocytes.[20,21] The hypothesis of an indirect effect of F on the rat erythrocyte Na^+-K^+-pump activity via suppression of energy exchange is confirmed by our earlier findings on the nucleated erythrocytes of frog, in which the energy supply for ion transport was provided solely by an oxidative phosphorylation - alternate pathway to produce ATP.[11] Treatment of the frog erythrocytes with 5 mM F did not affect an active transport of Rb^+ in the cells.[25] Besides, there are also data on the direct inhibitory influence of F on the activity of Na^+-K^+-ATPase.[5,7]

Although F and ouabain exert a similar effect on Rb^+ influx (Figure 1), the F-induced alterations in the cellular Na^+ and K^+ content are considerably greater than that induced by ouabain (Figures 2 and 3), suggesting that the influence of F is not limited by inhibition of the Na^+-K^+-pump. Apparently, F induces some additional effects on intracellular ion homeostasis dependent on the presence of Ca^{2+} in the medium. The F-induced accumulation of Na^+ in the cells is only partially diminished upon removal of Ca^{2+} from the incubation medium. The Ca^{2+}-independent alteration of Na^+ transport is most likely associated with a well-known capacity of F to suppress the activity of protein phosphatases.[26-28] This conclusion is confirmed by our recent work[24] establishing the similar stimulation of Na^+ influx into rat erythrocytes by F and selective inhibitors of types PP1 and PP2A protein phosphatases. Inhibition of the F-induced activation of Na^+ influx into rat erythrocytes by amiloride and bumetanide (Figure 8) indicates that F affects Na^+/H^+ exchange and/or Na^+ channels, as well as Na-K-2Cl cotransport. Such Ca^{2+}-independent activation of Na^+ transport through Na^+/H^+ exchange and Na-K-2Cl cotransport has been demonstrated in different cell types including erythrocytes.[8-10,29-31] In rat erythrocytes, these transport pathways are known to be stimulated under hyperosmotic shrinkage.[23,32] Evidently the erythrocytes shrink under the influence of F since the loss of cellular K^+ (and therefore, water) is not compensated by accumulation of Na^+ in the cells (Figures 2 and 3). Thus, alongside with an activation of Na^+ transport via inhibition of protein phosphatases, F can also enhance Na^+ influx into erythrocytes indirectly as a result of their shrinkage. The latter mechanism is Ca^{2+}-dependent and developed to considerable extent during the second hour of incubation (Figure 7).

In contrast to accumulation of Na^+ in the rat erythrocytes, loss of the cellular K^+ was completely dependent on the presence of Ca^{2+} in the incubation medium (Figure 5). F is able to increase the intracellular Ca^{2+} concentration ($[Ca^{2+}_i]$) in cell of various types.[15-18] Such effects of F on Ca^{2+} homeostasis in many cases can be attributed to the activation of G-proteins due to formation of its complexes with Al^{3+} rather than the action of F itself.[13,14,33,34] In the present work, however, we failed to find the evidence for involvement of G-proteins in the homeostasis of monovalent cations in rat erythrocytes. Our results agree with the data of Orlov and co-authors[35,36] who did not observe that Al^{3+} affects the activation of Na^+ and K^+ transport by F in rat erythrocytes.

Significant loss of K^+ by the cells under F exposure can also be explained by the increase of $[Ca^{2+}]_i$, causing the opening of K^+ channels.[37,38] There is much evidence that F increases $[Ca^{2+}]_i$ in a variety of cell types due to stimulation of Ca^{2+} influx[39,40] or inhibition of Ca^{2+}-ATPase activity.[6,41] Moreover, an increase of both $[Ca^{2+}]_i$ and F might influence ion transport through the cell membrane owing to alteration in the activity of protein phosphatases and protein kinases.[8-12,42] The decrease of F-induced Na^+ accumulation and K^+ loss in the presence of 1 mM Co^{2+} probably indicates the suppression of Ca^{2+} influx in the cells (Figure 6). An addition of Co^{2+} to the incubation medium is known to inhibit Ca^{2+} influx in rat and human erythrocytes.[43] Partial suppression of the F-induced Na^+ accumulation and K^+ loss (Figure 6) under influence of KN-93 indicates an involvement of Ca^{2+}/CaM-dependent protein kinase II (CaMKII) in the mechanism of F action. It is known, for example, that CaMKII participates in the activation of K^+ channels in rat endothelial cells under hypoxia.[44]

The nature of F-induced K^+ transport from the rat erythrocytes remains unclear. Quinine, an inhibitor of K^+ channels known to virtually completely suppress the activity of the Ca^{2+} activated K^+ channels in erythrocytes,[38,45] caused only a 16% decrease of the K^+ loss in the presence of F (Figure 6). On the other hand, application of amiloride induced a 30% reduction of the F-induced K^+ loss (Figure 6). Amiloride is widely used for the inhibition of different Na^+ transport pathways, but there are also data on its capacity to block certain types of K^+ channels.[46,47]

In conclusion, we found that F almost completely blocked the Na^+-K^+-pump, possibly due to the suppression of energy supply. Inhibition of protein phosphatases may cause an activation of Na^+ influx via an amiloride-sensitive pathway and to a lesser extent via Na-K-2Cl cotransport. These effects of F do not depend on the presence of Ca^{2+} in the incubation medium and are accompanied by relatively slow accumulation of Na^+ and loss of K^+. An additional stimulation of Na^+ influx in the cells in the presence of Ca^{2+} in the medium appears to be coupled with the decrease of cell volume due to loss of the cellular K^+. Substantial efflux of K^+ from the rat erythrocytes, observed only in the presence of Ca^{2+} in medium, is probably provided by the opening of Ca^{2+}-activated K^+ channels.

REFERENCES

1 Guminska M. Biochemical mechanisms of fluoride action on living organism. Folia Med Cracov 1981;23:305-21.

2 McIvor ME. Acute fluoride toxicity. Pathophysiology and management. Drug Saf 1990;5:79-85.
3 Vani ML, Reddy KP. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. Fluoride 2000;33:17-26.
4 Akdogan M, Eraslan G, Gultekin F, Sahindokuyucu F, Essiz D. Effects of fluoride on lipid peroxidation in rabbits. Fluoride 2004;37:185-9.
5 Yoshida H, Nagai K, Kamei M, Nakagawa Y. Irreversible inactivation of (Na^+-K^+)-dependent ATPase and K^+-dependent phosphatase by fluoride. Biochim Biophys Acta 1968;150:162-4.
6 Murphy AJ, Coll RJ. Fluoride is a slow, tight-binding inhibitor of the calcium ATPase of sarcoplasmic reticulum. J Biol Chem 1992;267:5229-35.
7 Kravtsova VV, Kravtsov OV. Inactivation of Na^+,K^+-ATPase from cattle brain by sodium fluoride. Ukr Biokhim Zh 2004;76:39-47.
8 Altamirano AA, Breitwieser GE, Russell JM. Vanadate and fluoride effects on Na^+-K^+-Cl^- cotransport in squid giant axon. Am J Physiol 1988;254:C582-6.
9 Pewitt EB, Hegde RS, Haas M, Palfrey HC. The regulation of Na/K/2Cl cotransport and bumetanide binding in avian erythrocytes by protein phosphorylation and dephosphorylation. Effects of kinase inhibitors and okadaic acid. J Biol Chem 1990;265:20747-56.
10 Lytle C. Activation of the avian erythrocyte Na-K-Cl cotransport protein by cell shrinkage, cAMP, fluoride, and calyculin-A involves phosphorylation at common sites. J Biol Chem 1997;272:15069-77.
11 Agalakova NI, Lapin AV, Gusev GP. Effects of fluoride and vanadate on K^+ transport across the erythrocyte membrane of *Rana temporaria*. Membr Cell Biol 2000;13:527-36.
12 Gusev GP, Ivanova TI. Activation of Na^+/H^+ exchange by protein phosphatase inhibitors in red blood cells of frog *Rana ridibunda*. J Comp Physiol 2003;173:429-35.
13 Strunecká A, Strunecký O, Patocka J. Fluoride plus aluminum: useful tools in laboratory investigations, but messengers of false information. Physiol Res 2002;51:557-64.
14 Suwalsky M, Norris B, Villena F, Cuevas F, Sotomayor P, Zatta P. Aluminum fluoride affects the structure and functions of cell membranes. Food Chem Toxicol 2004;42:925-33.
15 Blackmore PF, Bocckino SB, Waynick LE, Exton JH. Role of a guanine nucleotide-binding regulatory protein in the hydrolysis of hepatocyte phosphatidylinositol 4,5-bisphosphate by calcium-mobilizing hormones and the control of cell calcium. Studies utilizing aluminum fluoride. J Biol Chem 1985; 260:14477-83.
16 English D, Akard LP, Taylor GS, Rizzo MT, Dominguez J, Garcia JG. Gp-regulated phosphoinositide hydrolysis in turkey and human erythrocytes exposed to fluoride ion: relationship to calcium influx. J Lab Clin Med 1992;119:87-98.
17 Hughes BP, Barritt GJ. The stimulation by sodium fluoride of plasma-membrane Ca^{2+} inflow in isolated hepatocytes. Evidence that a GTP-binding regulatory protein is involved in the hormonal stimulation of Ca^{2+} inflow. Biochem J 1987;245:41-7.
18 Fukao M, Hattori Y, Sato A, Liu MY, Watanabe H, Kim TQ, et al. Relationship between NaF- and thapsigargin-induced endothelium-dependent hyperpolarization in rat mesenteric artery. Br J Pharmacol 1999;126:1567-74.
19 Whittam R. Control of membrane permeability to potassium in red blood cells. Nature 1968;219:610.
20 Feig SA, Shonet SB, Nathan DG. Energy metabolism in human erythrocytes. I. Effects of sodium fluoride. J Clin Invest 1971;50:1731-7.
21 Millman MS, Omachi A. The role of oxidized nicotinamide adenine dinucleotide in fluoride inhibition of active sodium transport in human erythrocytes. J Gen Physiol 1972;60:337-50.
22 Orlov SN, Kuznetsov SR, Kolosova IA, Aksentsev SL, Konev SV. Volume-dependent regulation of ion carriers in human and rat erythrocytes: role of cytoskeleton and protein phosphorylation. Ross Fiziol Zh Im I.M.Sechenova 1997;83:119-47.
23 Kolosova IA, Bernhard I, Orlov SN, Builler FR. The role of GTP-binding proteins in regulating the activation of Na^+/H^+-exchange and Na^+, K^+, $2Cl^-$ cotransport: effect of fluoride ion. Biokhimiia 1993;58:456-60.
24 Ivanova TI, Agalakova NI, Gusev GP. Activation of sodium transport in rat erythrocytes by inhibition of protein phosphatases 1 and 2A. Comp Biochem Physiol 2006;145:60-7.
25 Agalakova NI, Lapin AV, Gusev GP. Differential effects of glycolytic and oxidative metabolism blockers on the Na-K pump activity in erythrocytes of the frog, Rana temporaria. J Comp Physiol B 1997;167:576-81.

26 Shacter-Noiman E, Chock PB. Properties of a Mr = 38,000 phosphoprotein phosphatase. Modulation by divalent cations, ATP, and fluoride. J Biol Chem 1983;258:4214-9.
27 Kiener PA, Carroll D, Roth BJ. Westhead, E.W. Purification and characterization of a high molecular weight type 1 phosphoprotein phosphatase from the human erythrocyte. J Biol Chem 1987;262:2016-24.
28 Brautigan DL, Shriner CL. Methods to distinguish various types of protein phosphatase activity. Methods Enzymol 1988;159:339-46.
29 Pedersen SF, King SA, Rigor RR, Zhuang Z, Warren JM, Cala PM. Molecular cloning of NHE1 from winter flounder RBCs: activation by osmotic shrinkage, cAMP, and calyculin A. Am J Physiol 2003;284:C1561-76.
30 Romero M, Guizouarn H, Pellissier B, Garcia-Romeu F, Motais R. The erythrocyte Na^+/H^+ exchangers of eel (*Anguilla anguilla*) and rainbow trout (*Oncorhynchus mykiss*): a comparative study. J Exp Biol 1996;199:415-26.
31 Krarup T, Jakobsen LD., Jensen BS, Hoffmann EK. Na^+-K^+-$2Cl^-$ cotransport in Ehrlich cells: regulation by protein phosphatases and kinases. Am J Physiol 1998;275:C239-50.
32 Gusev GP, Ivanova TI. Activation of Na^+/H^+ exchange and Na^+, K^+, $2Cl^-$ cotransport after hyperosmotic shrinkage of the rat erythrocytes. Biol Membr 2005;22:72-8.
33 Himpens B, Missiaen L, Droogmans G, Casteels R. AlF^{4-} induces Ca^{2+} oscillations in guinea-pig ileal smooth muscle. Pflugers Arch 1991;417:645-50.
34 Matzel LD, Rogers RF, Talk AC. Bidirectional regulation of neuronal potassium currents by the G-protein activator aluminum fluoride as a function of intracellular calcium concentration. Neuroscience 1996;74:1175-85.
35 Orlov SN, Kolosova IA, Cragoe EJ, Gurlo TG, Mongin AA, Aksentsev SL, et al. Kinetics and peculiarities of thermal inactivation of volume-induced Na^+/H^+ exchange, Na^+,K^+,$2Cl^-$ cotransport and K^+,Cl^- cotransport in rat erythrocytes. Biochim Biophys Acta 1993;1151:186-92.
36 Orlov SN, Sen CK, Kolosova IA., Bernhardt J, Hänninen O, Bühler FR. Evidence for the involvement of G proteins in the modulation of ^{22}Na and ^{45}Ca fluxes in myogenic L6 and aortic smooth muscle cells. Biochem Biophys Res Commun 1993;191:802-10.
37 Burgess GM, Claret M, Jenkinson DH. Effects of quinine and apamin on the calcium-dependent potassium permeability of mammalian hepatocytes and red cells. J Physiol, 1981;317:67-90.
38 Crespo LM, Novak TS, Freedman JC. Calcium, cell shrinkage, and prolytic state of human red blood cells. Am J Physiol, 1987;252:C138-52.
39 Narayanan N, Su N, Bedard P. Inhibitory and stimulatory effects of fluoride on the calcium pump of cardiac sarcoplasmic reticulum. Biochim Biophys Acta 1991;1070:83-91.
40 Nijjar M, Belgrave RL. Regulation of Ca^{2+} homeostasis by glucose metabolism in rat brain. Mol Cell Biochem 1997;176:317-26.
41 Reis M, Farage M, de Souza AC, de Meis L. Correlation between uncoupled ATP hydrolysis and heat production by the sarcoplasmic reticulum Ca^{2+}-ATPase: coupling effect of fluoride. J Biol Chem 2001;276:42793-800.
42 Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling, Nat Rev Mol Cell Biol 2000;1:11-21.
43 Engelmann B, Duhm J. Distinction of two components of passive Ca^{2+} transport into human erythrocytes by Ca^{2+} entry blockers. Biochim Biophys Acta 1989;981:36-42.
44 Balla Z, Hoch B, Karczewski P, Blasig IE. Calcium/calmodulin-dependent protein kinase IIdelta 2 and gamma isoforms regulate potassium currents of rat brain capillary endothelial cells under hypoxic conditions. J Biol Chem 2002;277:21306-14.
45 Reichstein E, Rothstein A. Effects of quinine on Ca^{++}-induced K^+ efflux from human red blood cells. J Membr Biol 1981;59:57-63.
46 Discala F, Hulin P, Belachgar F, Planelles G, Edelman A, Anagnostopoulos T. Millimolar amiloride concentration block K conductance in proximal tubular cells. Br J Pharmacol 1992;107:532–8
47 Kirk K. K^+ transport across the lamprey erythrocyte membrane: characteristics of a $Ba(^{2+})$- and amiloride-sensitive pathway. J Exp Biol 1991;159:303-24.