

PHARMACOLOGICAL AND TOXICOLOGICAL EFFECTS OF ALUMINOFLUORIDE COMPLEXES

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SUMMARY: Laboratory investigations have often used aluminofluoride complexes for stimulation of various guanine nucleotide binding proteins. These complexes form spontaneously in aqueous solutions containing fluoride and traces of aluminum and appear to act as phosphate analogs. In view of the ubiquity of phosphate in cell metabolism and together with the dramatic increase in the amount of reactive aluminum now found in ecosystems, aluminofluoride complexes represent a strong potential danger for living organisms including humans. Although the possibility of pathophysiological consequences of their long-term action are not yet fully recognized, the pharmacological and toxicological effects of aluminofluoride complexes on animal and human cells, tissues, and organs are identified and summarized in this review.

Keywords: Aluminum, Fluoride, Aluminofluoride complexes, G proteins, Toxicological effects of Al-F.

INTRODUCTION

Fluoride anions have long been known to influence the activity of a variety of enzymes. Laboratory investigations have often used fluoride activation for stimulation of guanine nucleotide binding proteins (G proteins).¹ As reported by others, fluoride activation of adenylate cyclase depends on traces of aluminum.² The requirement for aluminum is highly specific: of 28 other metals tested, only beryllium promoted activation of the guanine nucleotide-binding regulatory component of adenylate cyclase by fluoride.

G proteins. Knowledge about the role of G proteins in signal transduction has expanded enormously during the last decade, as over one hundred G protein-coupled receptors have been described.^{1,3} G proteins couple membrane-bound heptahelical receptors to their cellular effector systems. The members of the G protein family are heterotrimeric proteins composed of α , β , and γ subunits. The G protein α subunit binds to GDP in the inactive state. The agonist receptor binding facilitates the exchange of GDP for GTP. The activated α subunit dissociates from γ and β to interact with effector enzymes such as adenylate cyclase or phospholipase C. Low K_M GTPase intrinsic activity of the α subunit hydrolyzes GTP to GDP to end the cycle activation.^{1,3} Molecular cloning has revealed the diversity of G proteins. At least 20 α , 5 β , and 7 γ subunits have already been identified.³

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A list of abbreviations used in this paper: AD, Alzheimer's disease; $[AlF_4]^-$, aluminofluoride complexes; cAMP, cyclic adenosine monophosphate; G proteins, guanine nucleotide binding proteins; GDP, guanosine diphosphate; GTP, guanosine triphosphate.

Aluminofluoride complexes. Low cost and availability of aluminum and fluoride salts have probably contributed to the fact that they are now widely used in laboratory studies of G proteins. Aluminofluoride complexes are formed spontaneously in a water solution containing fluoride and trace amounts of aluminum. The complexes are not permanent; equilibria exist between the various possible complexes, and the proportions of multifluorinated species such as AlF_3 , $\text{AlF}_3(\text{OH})^-$ and $[\text{AlF}_4]^-$, depend on the excess concentration of free F^- ions and on the pH of the solution.^{4,5} However, the exact structures of the activatory complexes are still disputed.

In aqueous solutions with a pH of less than 3, aluminum exists as the $\text{Al}(\text{H}_2\text{O})_6^{3+}$ ion, usually abbreviated to Al^{3+} . In a less acid water, $\text{Al}(\text{H}_2\text{O})_6^{3+}$ undergoes successive deprotonation, becoming $\text{Al}(\text{H}_2\text{O})_5(\text{OH})^{2+}$. Minimum solubility occurs in neutral solutions with the precipitation of $\text{Al}(\text{OH})_3$.⁶ In more basic solution, this solid redissolves, due to the formation of tetrahedral $\text{Al}(\text{OH})_4^-$. The fluoride anions bind to metal cation and are exchangeable with free fluoride or hydroxyl ions.

In millimolar fluoride and micromolar amounts of aluminum, $[\text{AlF}_4]^-$ was suggested to be the active species.^{5,7} The high concentration of fluoride anion in the solution induces the formation of a soluble tetracoordinated state of aluminum. According to models of Bigay *et al.*,⁵ it is unlikely that the whole $[\text{AlF}_4]^-$ complex binds with its four fluoride ions. Most probably, when entering the binding site, one of the fluoride ions is released and the tetracoordinated aluminum binds to the oxygen on the β phosphate. The aluminum is tetrahedrally bound to three fluorides and to the oxygen of the β phosphate of a dinucleotide.

Martin⁶ recalculated the equilibrium of aluminofluoride complexes and suggested that the predominant species are the neutral complex AlF_3 and the anionic mixed complex $\text{AlF}_3(\text{OH})^-$. These complexes should be hexacoordinated, with water molecules occupying the free sites. Only the hydroxylated and the ternary fluorohydroxylated complexes would be tetrahedral. For aluminum, it is uncertain whether the complex that enters the site is an AlF_3 that becomes tetrahedral by loosing its three bound water molecules and contracting a fourth bond with the β phosphate oxygen, or if it is already a tetrahedral $\text{AlF}_3(\text{OH})^-$ that exchanges its hydroxyl for the β phosphate oxygen or an $[\text{AlF}_4]^-$ that exchanges a fluorine. It seems, however, that the bound aluminum is tetracoordinated and trapped in the site with three fluorides.⁶ Further studies demonstrated that slow equilibration kinetics between various aluminofluoride complexes could give rise to puzzling kinetics that had caused misinterpretation of results.⁸ Once corrected for these effects, Antony and Chabre⁸ suggest that $\text{AlF}_3(\text{OH})^-$ is the main activating species and that the bound form of the complex is tetracoordinated GDP-AlF_3 .

Aluminofluoride complexes – new phosphate analogs. Bigay *et al.*⁵ demonstrated that $[\text{AlF}_4]^-$ activates stoichiometric amounts of transducin (the G protein of the vertebrate photoreceptor cells), with an affinity in the micromolar range. They found that this activation requires the presence of GDP. The acti-

vation of a transducin α subunit by $[\text{AlF}_4]^-$ does not require the presence of β or γ subunits, nor catalysis by an activated receptor. These authors suggested that $[\text{AlF}_4]^-$ acts as a high affinity analog of the γ phosphate.^{5,7} $[\text{AlF}_4]^-$ mimics the role of the γ phosphate only if the β phosphate is present and remains unsubstituted. The effect is more readily seen with G proteins because GDP is always tightly bound at the site after the hydrolysis of GTP.

The idea that an aluminofluoride complex acts as an analog of the terminal phosphate of GTP was proposed because the Al-F bond length is close to the P-O phosphate bond length. Both structures are tetrahedral. Fluorine and oxygen have nearly the same size and the same valence orbitals, but fluorine is more electronegative and has an even greater capacity than oxygen for forming hydrogen bonds. Aluminum is close to phosphorus in the periodic table, and their valence electrons are in the same third shell. A high concentration of fluoride anions in solution induces the formation of a soluble tetracoordinated state of aluminum, which has the same geometry, size, and coordination as phosphate.

Chabre⁷ explained an important "functional" difference between a phosphate group and the structurally analogous aluminofluoride complexes. In phosphate, oxygen is covalently bound to the phosphorus and does not exchange with oxygen from solvent. In aluminofluoride complexes, the bonding between the electropositive aluminum and the highly electronegative fluorine is more ionic in character. The reaction of a bound phosphate compound with orthophosphate is endergonic and slow, whereas the corresponding reaction with an aluminofluoride complex is rapid and spontaneous. Fluorides in the bound complexes can also exchange with free fluoride ions in solution.

Fluoride activation is used in laboratory investigations as evidence for involvement of a G protein in a system. Aluminofluoride complexes mimic the action of many neurotransmitters, hormones, and growth factors. Aluminofluoride complexes also affect the activity of a variety of phosphatases, phosphorylases, and kinases.^{5,7} Studies which utilize aluminofluoride complexes in laboratory investigations and show the effects of fluoride on various cells and tissues in the presence of aluminum will now be reviewed.

PHYSIOLOGICAL AND BIOCHEMICAL ACTION OF ALUMINOFLUORIDE COMPLEXES IN VARIOUS CELLS AND TISSUES

Liver. Isolated liver parenchymal cells, hepatocytes, maintain responsiveness to hormones and serve as model cells equipped with very complex biochemical pathways. The stimulation of glycolysis by vasopressin, angiotensin II, and alpha-1-adrenergic agonists is mediated in the liver through the increase of the cytosolic Ca^{2+} level. It has been demonstrated that the phosphoinositide signaling second messenger system is activated and involved in these events. Blackmore *et al*^{9,10} demonstrated in their studies that the treatment of isolated hepatocytes with NaF produced an efflux of Ca^{2+} and a rise in free cytosolic Ca^{2+} . Alterations in the phosphoinositide signaling system were observed, such as: the decrease in phosphatidylinositol 4,5-bis-phosphate content and the increase in the level of inositol-1,4,5-trisphosphate. The level of intracellular cyclic adenosine monophosphate (cAMP) was decreased. All these changes were

concentration dependent. AlCl_3 potentiated the effects of low doses of NaF (2-15 mM), and this potentiation was abolished by the Al^{3+} chelator desferoxamine. Fluoride anions in the presence of aluminum thus mimicked the action of Ca^{2+} -mobilizing hormones glucagon and vasopressin in hepatocytes. The effects of submaximal doses of fluoride salts were potentiated by submaximal doses of vasopressin, angiotensin II, and α_1 -adrenergic agonists. Using phorbol myristate acetate, the activator of protein kinase C, it was determined that aluminofluoride complexes mimic the effects of Ca^{2+} -mobilizing hormones by activating the G protein which couples the hormone receptor to phospholipase C specific to phosphatidylinositol 4,5-bis-phosphate.¹¹ Blackmore *et al*⁹ also observed the activation of phosphorylase and inactivation of glycogen synthase in the presence of fluoride and AlCl_3 in hepatocytes.

Fluoride anions in the presence of aluminum thus affect the liver as an organ involved in glycogenolysis, fatty acid oxidation, and lipolysis.

Brain. G protein-mediated cell responses are of key importance in the processes of neurotransmission and intercellular signaling in the brain.¹² Phosphoinositide metabolism is coupled to several neurotransmitter receptors in the central nervous system including cholinergic, adrenergic, dopaminergic, and histaminergic receptors. Aluminofluoride complexes have been widely used to stimulate phosphoinositide hydrolysis. The ability of fluoride in the presence of trace amounts of aluminum to mimic the effects of Ca^{2+} -mobilizing hormones suggests the coupling of hormone receptors to phosphoinositide breakdown through G proteins.¹²

Candura *et al*¹³ observed that aluminum salts and NaF mimicked the action of GTP (S) in stimulating phosphoinositide turnover and generation of inositol phosphates in rat cerebral cortical membranes. A much greater hydrolysis of phosphoinositides was observed when AlCl_3 and NaF were present together, supporting the concept that $[\text{AlF}_4]^-$ is the active stimulatory species. Nadakavukaren *et al*¹⁴ demonstrated accumulation of inositol phosphates in the suprachiasmatic nuclei region of rat hypothalamus over a 40-min incubation with aluminum fluoride. Hypothalamic suprachiasmatic nuclei were suggested as the site of a biological clock responsible for generation of circadian rhythms.

Brief exposure to aluminofluoride complexes induced prolonged enhancement of synaptic transmission in rat hippocampal slices.¹⁵ When rat hippocampal slices were exposed to 10 mM NaF and 10 μM AlCl_3 for a brief period of time (12-15 min), spike amplitude fell to very low levels. Upon washout, spike amplitude recovered beyond control values and in half of the preparations a prolonged enhancement of spike amplitude (greater than 2 hours) occurred. If AlCl_3 was omitted from fluoride-containing saline, enhancement of spike amplitude, when observed, was brief.

Enormous possibilities for multiple molecular interactions of aluminum and fluoride exist in the brain and clearly warrant further investigation.

Kidney. The effects of aluminofluoride complexes on the kidney have been studied using glomerular mesangial cells, proximal tubular cells, and inner me-

dullar collecting tubule cells of rat kidney. Fluoride and aluminum in kidney tubular cells affect the ion transporting processes. Aluminofluoride complexes stimulate adenylate cyclase, inhibit amiloride-sensitive Na/H exchange regulated by cAMP-dependent protein kinase, enhance epidermal growth factor-stimulated prostaglandin production, and mimic vasopressin and bradykinin induced Ca^{2+} mobilization. It is suggested that aluminofluoride complexes can affect the activity of many other ion channels and enzymes in the kidney.¹⁶

AlF_3 or NaF at various concentrations was given in the drinking water for 45 weeks.^{17,18} Pathological changes were found in the kidneys of all groups. Aluminum-containing deposits were found in the kidney blood vessels, and the renal aluminum content was doubled when the rats drank the AlF_3 water. The kidneys from rats drinking the NaF water exhibited glomerular hypercellularity, renal mesangial proliferation, and the deposition of proteins in the renal tubules.^{17,18} Histological evidence of glomerular distortions was present in both the AlF_3 and NaF groups.

Blood Cells. Incubation of platelets with NaF (5-10 mM) induced only slight morphological changes. Addition of 10 μM AlCl_3 resulted in platelet aggregation.¹⁹ One min after addition of AlCl_3 , most of the granules were concentrated in the center of the cell, but some cells were extruding their contents by direct exocytosis. No myosin light-chain phosphorylation typical for the platelet response was observed after fluoride activation in the presence of aluminum. It has been reported that aluminofluoride complexes impair the polymerization-depolymerization cycle of tubulin.⁵

Rapid and dynamic changes of the actin network are of vital importance for the motility of human neutrophils. Bengtsson *et al*²⁰ observed $[\text{AlF}_4]^-$ induction of a pronounced and sustained increase in a filamentous form of actin in intact human neutrophils. This effect parallels an increase in cytosolic Ca^{2+} level, indicating that phospholipase C is activated. Shape changes and disorganization of the spectrin network were observed after addition of 1 mM NaF and 10 μM AlCl_3 in human red blood cells.²¹ Cells lost their membrane material and became smaller.

Osteoblasts and osteoclasts. Bone matrix is secreted by osteoblasts that lie at the surface of the existing matrix. On the other hand, bone matrix is eroded by osteoclasts. The hormone calcitonin inhibits osteoclastic bone resorption. The activation of calcitonin involves two separate effects on the osteoclast: abolition of cell motility and marked cellular retraction. Cell motility is mimicked by dibutyryl cAMP and by cholera toxin. Meanwhile, pertussis toxin and increase in ambient Ca^{2+} mimic cellular retraction. $[\text{AlF}_4]^-$ produces both effects. Caverzasio *et al*²² found that traces of aluminum markedly enhanced the stimulation of inorganic phosphate transport induced by fluoride in osteoblasts, suggesting that an aluminofluoride complex might be responsible for a fluoride-induced regulatory pathway. Analysis of the role of tyrosine phosphorylation in mediating this cellular response indicates that this signal transduction pathway is also involved in the stimulation of inorganic phosphate transport activity by fluoride. Aluminum potentiates the effect of fluo-

ride on tyrosine phosphorylation and osteoblast replication *in vitro* and bone mass *in vivo*. The combination of fluoride and aluminum modulates a growth factor-dependent tyrosine kinase pathway enhancing mitogen-activated protein kinase and osteoblastic proliferation. Low doses of fluoride stimulate the recruitment and lifespan of osteoblasts; at higher doses, fluoride decreases osteoblast activity.²³ Exposure of osteoclasts to $[\text{AlF}_4]^-$ resulted in a marked inhibition of bone resorption.²⁴

Laboratory experiments have therefore demonstrated that exposure of osteoclasts and osteoblasts to aluminofluoride complexes markedly affects bone formation. The results suggest an involvement of G proteins in these processes and indicate that fluoride and aluminum may alter osteoclast and osteoblast behavior.

Fibroblasts. In a living organism, fibroblasts must be able to move into areas of newly forming tissue and to secrete molecules that help glue the tissue together. Laboratory investigations clearly indicate that both the production of extracellular matrix and cell movement can be affected by the action of aluminofluoride complexes. Stimulation of fibroblasts by hormones linked to the phosphoinositide signaling system elicits oscillation of cytosolic Ca^{2+} concentration. Such oscillation waves are linked to fluctuations in the concentrations of inositol-1,4,5-trisphosphate and the Ca^{2+} content of intracellular inositol-1,4,5-trisphosphate-sensitive Ca^{2+} stores. Ca^{2+} oscillations in REF52 fibroblasts can also be generated by direct stimulation of G proteins with aluminofluoride complexes.²⁵ Oguro *et al*²⁶ studied the cytotoxicity of NaF on fibroblast-like cells from five Japanese whole fetuses and found that the growth of the cells was markedly impaired by fluoride.

Apoptosis. Loweth *et al*²⁷ showed that fluoride induces apoptosis in clonal pancreatic beta cells and in the cells of normal rat islets of Langerhans. The process may reflect the formation of $[\text{AlF}_4]^-$ since it was inhibited by the aluminum chelator deferoxamine. Recent studies provide evidence that apoptosis of pancreatic β cells is important in the early etiology of diabetes mellitus. Treating thymus lobe cells with aluminofluoride complexes also provoked apoptosis of a wider range of thymocyte subtypes²⁸ with an accumulation of inositol phosphates. The responses to aluminofluoride complexes were not prevented by inhibitors of tyrosine kinases, suggesting that unidentified G proteins which couple to phospholipase C activation may also be capable of initiating apoptosis by a route independent of the T cell receptor.

Energy metabolism. ATP generation in mitochondria requires the association of F_1 subunit with F_0 transmembrane subunit transporting protons. The binding of ADP and inorganic phosphate in a catalytic site of F_1 triggers conformational changes, which lock both of them into the site and induce the formation of pyrophosphate bonds by eliminating a water molecule.⁷ Lunardi *et al*²⁹ reported the inhibition of mitochondria ATPase activity in the presence of $[\text{AlF}_4]^-$. This inhibition is not reversed by elution of fluoride from solution or by the addition of strong aluminum chelators. No significant release of the complex occurred over a period of days. Aluminofluoride complexes inhibit

many ATPases, phosphatases, and phosphorylases. The intervention of aluminofluoride complexes in the energy transformation processes may thus affect the energy metabolism of the entire organism.

What do these laboratory investigations tell us? Laboratory investigations support the hypothesis that G proteins are potential fluoride and aluminum targets. It might seem difficult to decide if numerous laboratory experiments demonstrate a potential toxicological risk of fluoride for the human population. Fluoride in the presence of aluminum acts as the initial signal triggering processes of neurotransmission and potentiating the action of various hormones. The initial signal is greatly amplified during its conversion into the functional response. Aluminofluoride complexes influence all types of cells and tissues of the human body with powerful pharmacological effects. Fluoride in the presence of trace amounts of aluminum affects blood elements and cells of the immune system, protein phosphorylation and organization of cytoskeletal proteins, the functions of bone cells, processes of calcium homeostasis, ion transport, and energy metabolism.

It is surprising that numerous laboratory findings of adverse effects of fluoride in the presence of aluminum have not been reported until recently.³⁰ Even though the pathophysiological consequences of the long-term action of aluminofluoride complexes are still not fully recognized, the implications of laboratory investigations using isolated animal and human cells or tissues on the intact human organism can be discussed.

EVIDENCE FOR INVOLVEMENT OF ALUMINUM, FLUORIDE, AND ALUMINOFLUORIDE COMPLEXES IN PATHOLOGY

Aluminum and fluoride intoxication in chronic hemodialysis patients. Elevated aluminum levels have been implicated as the cause of dialysis encephalopathy or dementia in renal failure patients after three to seven years of hemodialysis treatment.^{31,32} Speech disorders precede dementia and convulsions. The mode of death has been reported as sudden cardiac arrest usually associated with acute pulmonary edema.³¹

Increased serum fluoride concentration and fluoride intoxication have also been observed in chronic hemodialysis patients.³³ Arnow *et al*³⁴ reported that 12 of 15 patients receiving dialysis treatment in one room became acutely ill, with severe pruritus, multiple nonspecific symptoms, and/or fatal ventricular fibrillation (3 patients). Death was associated with longer hemodialysis time and increased age compared with other patients who became ill. Serum concentrations of fluoride in the sick patients were markedly increased to as high as 716 μM . The source of fluoride was the faulty temporary deionization system used to purify water for hemodialysis.

Occupational fluoride exposure. Soyseth *et al*³⁵ investigated the relation between plasma fluoride levels and bronchial responsiveness in a longitudinal study in aluminum potroom workers who reported work-related asthmatic symptoms. A positive association was found between bronchial responsiveness and plasma fluoride levels. Osteoarthritis and related disorders in such workers have been reported since the 1930s.³⁶

Psychiatric disturbances have been reported in aluminum smelter workers.^{37,38} Persons living near an enamel factory that emitted hydrogen fluoride into the air had a distinct decline in mental acuity, memory loss, inability to coordinate thoughts, and reduced ability to write.

Alzheimer's disease. Because a higher post-mortem level of aluminum has been found in the brains of people with Alzheimer's disease (AD) than in the brains of age-matched healthy controls, the hypothesis linking the accumulation of toxic amounts of aluminum in the brain with Alzheimer's dementia has often been proposed.³⁹⁻⁴¹ A positive correlation between the incidence of AD and concentrations of aluminum in drinking water has been reported by some authors.^{42,43} Neither the increased content of aluminum in the brain nor the results of ecological studies can explain why aluminum constitutes a risk. Aluminum is currently regarded as the putative risk factor for the etiology of this disease.

Recent fundamental research on the pathogenesis of AD has indicated that the disease is connected with alterations in neurotransmission, beta-amyloid production, plaque formation, and cytoskeletal abnormalities in brain tissue. The multiple effects which accompany AD demonstrate the diversified and multidimensional nature and integration of the nervous system. We suggest that some of the pathologic changes are not raised by aluminum alone, but also by aluminofluoride complexes.⁴⁴ However, aluminofluoride complexes may act as the initial signal that stimulates impairment of homeostasis, degeneration, and death of the cells. By influencing energy metabolism these complexes can accelerate aging and impair the functions of the nervous system. With respect to the etiology of AD, the long-term action of aluminofluoride complexes may represent a serious and powerful risk factor for the development of this devastating disease.

Bone formation. Osteosclerosis in workers exposed to fluoride and aluminum (industrial fluorosis) has led to the use of fluoride as a treatment to increase bone mass in osteoporosis patients. NaF is used clinically as a potent stimulator of bone formation. However, there are conflicting reports on the effect of fluoride on trabecular bone formation and bone strength. Of the 15 animal studies reviewed, reduced bone strength was found in seven studies.⁴⁵ Most of the evidence on fluoride and bone fracture comes from ecological studies of hip fracture, and the results have been inconsistent.⁴⁵ The problem of what evidence is sound and what is not has been discussed recently.⁴⁶ On this point, there appears to be a basic agreement that in some circumstances fluoride can contribute to hip fracture.

Aluminum-induced neural degeneration in rats is greatly enhanced when the animals were fed low doses of fluoride. The presence of fluoride caused more aluminum to cross the blood-brain barrier and be deposited in the brain.¹⁷ The reduction of neuronal density in the neocortex was more prominent in the AlF₃ group than in the NaF and control groups. Long-term ingestion of aluminum fluoride by rats causes damage to neuronal brain cells. The pathological changes found in the brain tissue of the animals given aluminum and fluoride

were similar to the alterations found in the brains of Alzheimer's disease patients. Experiments with rats showed that the toxicity of 0.5 ppm AlF_3 (as Al^{3+}) in the drinking water was significantly greater than 5 or even 50 ppm AlF_3 (as Al^{3+}).^{17,18} Possible cellular mechanisms which might underlie the association between the effect of aluminum and fluoride and regional patterns of neuronal injury include calcium homeostasis, secondary messenger systems, alterations in neuronal cytoskeleton, and alterations in cerebrovasculature.¹⁷

Fluoride-induced cardiopulmonary dysfunction. Fluoride infusion (0.9 mol/L in 0.9% NaCl for 3 h i.v.) in the presence and absence of AlCl_3 ($0.6 \mu\text{g}/\text{kg}^{-1}/\text{min}^{-1}$) into pigs anaesthetized with pentobarbital sodium was used.⁴⁷ NaF, with or without AlCl_3 , induced progressive deterioration of cardiopulmonary function after 1 h of infusion. At 3 h, mean pulmonary arterial pressure, pulmonary vascular resistance, tracheal pressure, and plasma concentrations of thromboxane B₂, 6-ketoprostaglandin F₁ α , and prostaglandin F₂ α were significantly increased to approximately 200, 520, 175, 759, 402, and 336%, respectively, of baseline values (0 h). At 3 h, cardiac index and arterial pO_2 decreased 38% and 28 Torr, respectively, from baseline values.

Diabetes. The hypothesis that people who suffer from diabetes mellitus may ingest abnormal levels of fluoride has been suggested.⁴⁸ Rats given 20 ppm fluoride in drinking water for 32 days show a decrease in red blood cells and haematocrit. The greater toxicity of fluoride in alloxan-induced diabetic rats in comparison with control group was also observed.⁴⁹ NaF (1-20 mM) in the presence of 10 μM AlCl_3 produced slowly developing, concentration-dependent contractions in mesenteric arteries from three-month old streptozotocin-diabetic (60 mg/kg, i.v.) male Wistar rats and age-matched control rats.⁴⁹ The maximum contractile response was significantly greater in mesenteric arteries in diabetic than in control rats, as was the response to noradrenaline. Maximum contractile responses of aorta and caudal artery in diabetic rats to NaF were also significantly enhanced. These experiments show that aluminofluoride complexes enhanced contractile responses of these arteries to α_1 -adrenoceptor stimulation.

CONCLUSIONS

Aluminofluoride complexes appear to be a new class of phosphate analogs for laboratory investigations. Experimental data clearly indicate that aluminofluoride complexes stimulate various G proteins. These metallofluoride complexes may thus mimic or potentiate the action of numerous extracellular signals and significantly affect many cellular responses. The principle of amplification of the initial signal during its conversion into the functional response has been a widely accepted tenet in cell physiology. Fluoride ions in the presence of trace amounts of aluminum may therefore act with powerful pharmacological effects.

The results of laboratory investigations using isolated animal and human cells or tissues must be integrated into the functional whole. At present, this task is extremely difficult even for scientists. No one can predict exactly what happens in the human body. The natural barrier systems, such as low alumi-

num absorption in the gastrointestinal tract, and various physiological ligands, such as transferrin, citrate, phosphate, and silicic acid, are efficient buffers preventing the increased intake of this metal under natural conditions.⁵⁰

With the appearance of acid rain and due to the widespread use of aluminum in industry, there has been a dramatic increase in the amount of reactive aluminum appearing in ecosystems, food, and water sources.⁵¹⁻⁵³ Together with the increase of fluorides now in the environment and food chain, the possibility exists that the near future will supply us with more data about the danger of fluoride and trace amounts of aluminum for the human race. Although many epidemiological studies about the detrimental effects of fluoride and aluminum have been published, whether fluorides and/or aluminum are the causative agents for numerous disturbances remains to be determined.⁵⁴

ACKNOWLEDGEMENT

This work was supported by the Grant Agency of Charles University, Prague (Grant No. 113/1998/BBio/PřF).

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