Table 5. A few studies on G-Proteins and Fluoride	
Physiol Res. 2002;51(6):557-64. **Fluoride plus aluminum: useful tools in laboratory investigations, but messengers of false information.** Strunecka A, Strunecky O, Patocka J Department of Physiology and Developmental Biology, Faculty of Sciences, Charles University, Czech Republic.	Aluminofluoride complexes (AlF(x)) form spontaneously in aqueous solutions containing fluoride and traces of aluminum ions and appear to act as phosphate analogs. These complexes have become widely utilized in laboratory investigations of various guanine nucleotide-binding proteins. Reflecting on many laboratory studies, a new mechanism of fluoride and aluminum action on the cellular level is being suggested. The long-term synergistic effects of these ions in living environment and their hidden danger for human health are not yet fully recognized. FULL STUDY
Neurosci Lett. 2004 Jul 1;364(2):86-9. **"Low" concentrations of sodium fluoride inhibit neurotransmitter release from the guinea-pig superior cervical ganglion.** Borasio PG, Cervellati F, Pavan B, Pareschi MC. Department of Biology, Section of General Physiology, University of Ferrara, Via L. Borsari 46, 44100 Ferrara, Italy.	The electrically evoked (1 Hz, 5 min) [3H] release was inhibited by "low" F− concentrations (1–2.5 mM), by the adenylyl cyclase blocker MDL 12330A (10 μM), alone and in combination with 1 mM NaF, and increased by 0.5 mM 8Br-cAMP, 100 μM forskolin and 0.5 mM 3-isobutyl-1-methylxantine. The role of G proteins and related second messenger system on the modulation of acetylcholine release from [3H]choline-preloaded guinea-pig superior cervical ganglion was investigated using the potent general activator NaF. … a NaF-sensitive G protein, linked to cAMP synthesis, is determinant for the inhibition of neurosecretion in this cholinergic synapse, involving Ca2+-dependent mechanisms. these data suggest an involvement of the inhibitory regulatory subunit of the cAMP system in inducing presynaptic inhibition by interaction with calcium-sensitive structures. FULL STUDY at Science Direct Abstract
Int J Dev Neurosci 1999 Jul;17(4):357-67 **Fluoride-induced depletion of polyphosphoinositides in rat brain cortical slices: a rationale for the inhibitory effects on phospholipase C.** Sarri E, Claro E. Departament de Bioquimica i de Biologia Molecular, Facultat de Medicina, Universitat Autonoma de Barcelona, Spain.	Fluoride, which is used commonly as a pharmacological tool to activate phosphoinositide-phospholipase C coupled to the heterotrymeric Gq/11 proteins, inhibited the phosphorylation of phosphatidylinositol (PtdIns) to polyphosphoinositides (PtdIns4P and PtdIns4,5P2) in membranes from rat brain cortex. our data show that fluoride, at a concentration similar to that used to stimulate directly Gq/11-coupled phospholipase C, effectively blocks the synthesis of phospholipase C substrates from PtdIns. Abstract

J Neurophysiol 81: 2095-2102, 1999 **Glutamate Controls the Induction of GABA-Mediated Giant Depolarizing Potentials Through AMPA Receptors in Neonatal Rat Hippocampal Slices** Sonia Bolea (1), Elena Avignone (2), Nicola Berretta (2), Juan V. Sanchez-Andres (1), Enrico Cherubini (2) 1. Departamento de Fisiologia, Instituto de Bioingenieria, Universidad Miguel Hernandez, Campus de San Juan, 03550 San Juan, Alicante, Spain; 2. Neuroscience Program and Istituto Nazionale Fisica della Materia Unit, International School for Advanced Studies, 34014 Trieste, Italy	Intracellular blockade of GABA receptors with fluoride reveals a novel component of evoked GDPs FULL STUDY
J Neurosci 1996 Oct 1;16(19):5914-22. **Cholinergic stimulation of AP-1 and NF kappa B transcription factors is differentially sensitive to oxidative stress in SH-SY5Y neuroblastoma: relationship to phosphoinositide hydrolysis.** Li X, Song L, Jope RS. Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham 35294-0017, USA.	Oxidative stress appears to contribute to neuronal dysfunction in a number of neurodegenerative conditions, notably including Alzheimer's disease, in which cholinergic receptor-linked signal transduction activity is severely impaired. activation with NaF of G-proteins coupled to phospholipase C was concentration dependently inhibited by H2O2, indicating impaired G-protein function. These effects of H2O2 are similar to signaling impairments reported in Alzheimer's disease brain, which involve deficits in receptor- and G-protein-stimulated phosphoinositide hydrolysis, but not phospholipase C activity.
Continues …	

Exp Brain Res. 1995; 106(3):505-8. **Attenuation of high-voltage-activated Ca2+ current run-down in rat hippocampal CA1 pyramidal cells by NaF.** Breakwell NA, Behnisch T, Publicover SJ, Reymann KG. Department of Physiology, Trinity College Dublin, Ireland.	Abstract Calcium currents in CA1 neurons from rat **hippocampu**s were studied with the whole-cell, patch-clamp technique. Under control conditions high-voltage-activated (HVA) calcium currents activated from membrane potentials of -80 mV and -40 mV underwent "run-down". The rate of run-down of the current activated from -40 mV was significantly attenuated by inclusion of the G-protein activator NaF (1 mM) in the pipette and also irreversibly attenuated by brief batch application of NaF (10 mM)... It is suggested that activation of guanine nucleotide-binding proteins by NaF leads to a long-lasting attenuation of HVA calcium current run-down in hippocampal CA1 cells.
Brain Res. 1993 Nov 26; 629(1):133-40. **Aluminum decreases muscarinic, adrenergic, and metabotropic receptor-stimulated phosphoinositide hydrolysis in hippocampal and cortical slices from rat brain.** Shafer TJ, Mundy WR, Tilson HA. Cellular and Molecular Toxicology Branch, US EPA, Research Triangle Park, NC	Abstract In both hippocampal and cortical slices… Stimulation of G-proteins with NaF (5-30 mM) resulted in accumulation of Ips (inositol phosphate) in hippocampal and cortical slices in the absence of added agonists. NaF (5-30 mM) plus 1 mM CARB produced increased accumulation of IPs over CARB or NaF alone.
Brain Res. 1993 Sep 17; 622(1-2):35-42. **Alterations in the activity of adenylate cyclase and high affinity GTPase in Alzheimer's disease.** Ross BM, McLaughlin M, Roberts M, Milligan G, McCulloch J, Knowler JT. Wellcome Neuroscience Group, Wellcome Surgical Institute & Hugh Fraser Neuroscience Labs., Glasgow UK.	Abstract The aim of this study was to assess the effect of Alzheimer's disease has on the functional integrity of several signal transduction proteins. The relative levels of the G-protein alpha subunits Gs alpha-L, Gs alpha-S, Gi alpha-2 and G(o) alpha were measured by western blotting and found to be unchanged in membranes prepared from Alzheimer-diseased frontal cortex or hippocampus compared to control brains. However the activity of the G-protein associated enzyme, high affinity GTPase, was found to be reduced in the frontal cortex (reduced by 25%) and by a similar magnitude in the **hippocampus** (reduced by 27%) of Alzheimer subjects. The same membrane preparations were also assayed for the activity of adenylate cyclase. Basal enzyme activity was not significantly altered in Alzheimer diseased **hippocampus**, but was markedly reduced (by 45%) in the frontal cortex. The ability of fluoride and aluminium ions to stimulate adenylate cyclase was not significantly changed in either brain region. This suggests that G-proteins, especially Gs, are still able to interact with this enzyme. These results indicate that although the presence of Alzheimer's disease does not significantly alter G-protein levels, changes have taken place in the overall activity of these proteins. However this alteration does not affect their ability to stimulate adenylate cyclase activity.

Exp Brain Res 1991; 84(3):680-4 **Brief exposure to the G-protein activator NaF/AlCl3 induces prolonged enhancement of synaptic transmission in area CAI of rat hippocampal slices.** Publicover SJ. School of Biological Sciences, University of Birmingham, Edgbaston, UK.	Abstract Rat hippocampal slices were exposed briefly (12-15 min) to AlF4- (10 mmol/l NaF, 10 mumol/l AlCl3). The effect on synaptic transmission in area CAI was measured using extracellular electrodes placed in the stratum pyramidale and stratum radiatum. During fluoride exposure, both spike and EPSP amplitude fell to very low levels. It is concluded that NaF/ACl3 exposure induces an LTP-like process by G-protein activation,
J Neurochem. 1991 Jan;56(1):44-51. **Sodium fluoride mimics the effect of prostaglandin E2 on catecholamine release from bovine adrenal chromaffin cells.** Ito S, Negishi M. Mochizuki-Oda N, Yokohama H, Hayaishi O. Department of Cell Biology, Osaka Bioscience Institute, Suita, Japan.	Abstract An examination of the involvement of a GTP-binding protein(s) in PGE receptor-induced responses by using NaF. In the presence of Ca2+ in the medium, NaF stimulated the formation of all three inositol phosphates, i.e., inositol monophosphate, bisphosphate, and trisphosphate, linearly over 30 min in a dose-dependent manner (15-30 mM). This effect on phosphoinositide metabolism was accompanied by an increase in cytosolic free Ca2+. NaF also induced catecholamine release from chromaffin cells, and the dependency of stimulation of the release on NaF concentration was well correlated with those of NaF-enhanced inositol phosphate formation and increase in cytosolic free Ca2+. Although the effect of NaF on PGE2-induced catecholamine release in the presence of ouabain was additive at concentrations below 20 mM, there was no additive effect at 25 mM NaF. Furthermore, the time course of catecholamine release stimulated by 20 mM NaF in the presence of ouabain was quite similar to that by 1 microM PGE2, and both stimulations were markedly inhibited by amiloride, with half-maximal inhibition at 10 microM. Pretreatment of the cells with pertussis toxin did not prevent, but rather enhanced, PGE2-induced catecholamine release over the range of concentrations examined. **These results demonstrate that NaF mimics the effect of PGE2 on catecholamine release from chromaffin cells** and suggest that PGE2-evoked catecholamine release may be mediated by the stimulation of phosphoinositide metabolism through a putative GTP-binding protein insensitive to pertussis toxin.
Brain Res 1990 Dec 24;537(1-2):93-101 **Multiple actions of fluoride ions upon the phosphoinositide cycle in the rat brain.** Tiger G, Bjorklund PE, Brannstrom G, Fowler CJ. University of Umea, Sweden.	It is concluded that fluoride ions inhibit agonist-stimulated inositol phospholipid breakdown via actions not only on G-proteins but also on phosphoinositide-specific phospholipase C substrate availability.

Eur J Pharmacol 1990 Jan 23;188(1):51-62 **Effect of monovalent ions upon G proteins coupling muscarinic receptors to phosphoinositide hydrolysis in the rat cerebral cortex.** Tiger G, Bjorklund PE, Cowburn RF, Garlind A, O'Neill C, Wiehager B, Fowler CJ. Dept. of Pharmacology, University of Umea, Sweden.	Evidence is presented to suggest that NaF affects the dephosphorylation of the formed [3H]inositol polyphosphates.
J Neurochem 1990 Apr;54(4):1130-5 **Modulation of gamma-aminobutyric acid release in cerebral cortex by fluoride, phorbol ester, and phosphodiesterase inhibitors: differential sensitivity of acetylcholine release to fluoride and K+ channel blockers.** Gardiner IM, de Belleroche J. Dept. of Biochemistry, Charing Cross and Westminster Medical School, London, England.	In this study, fluoride was used as a tool to investigate the involvement of G protein-coupled effector systems in the regulation of the depolarization-induced release of gamma-aminobutyric acid (GABA) from rat cerebral cortex. From these studies, it is concluded that GABA release in cerebral cortex is subject to regulation by G protein-linked effector systems that are distinct from those affecting the release of [3H]ACh in cerebral cortex.
Biochem Biophys Res Commun 1988: Sep 15;155(2):664-9 **Fluoride inhibits agonist-induced formation of inositol phosphates in rat cortex.** Godfrey PP, Watson SP. Dept. of Clinical Pharmacology, Radcliffe Infirmary, Oxford, United Kingdom.	Since fluoride ions are known to activate G-proteins in the concentrations used in this study, these results may indicate the existence of a novel G-protein linked to receptor inhibition of phospholipase C.
Continued …	

Brain Res Dev Brain Res. 2002 Jan 31;133(1):69-75. **Ontogenetic development of the G protein-mediated adenylyl cyclase signalling in rat brain.** Ihnatovych I, Novotny J, Haugyicoya R, Bourova L, Mares P, Svoboda P Department of Developmental Epileptology, Institute of Physiology, Academy of Sciences, Vijdenska 1083, 142 20 Prague 4, Czech Republic.	Abstract … future research has to be oriented to identification of potential negative regulators of AC in the course of brain development. Among these, the newly discovered group of GTPase activating proteins, RGS, appears to be of primary importance because these proteins represent potent negative regulators of any G protein-mediated signalling in brain.
Biochem J 1987 May 15;244(1):35-40 **Guanine nucleotide and NaF stimulation of phospholipase C activity in rat cerebral-cortical membranes. Studies on substrate specificity.** Litosch I. Department of Pharmacology, University of Miami School of Medicine, FL 33101.	results indicate that, in cerebral-cortical membranes, activation of phospholipase C by guanine nucleotides or fluoride directly increases a phospholipase C activity which specifically hydrolyses PIP2.
J Neurosci. 1986 Oct;6(10):2915-20. **Intracellular fluoride alters the kinetic properties of calcium currents facilitating the investigation of synaptic events in hippocampal neurons.** Kay AR, Miles R, Wong RK.	Authors attempted to suppress voltage-dependent conductances in hippocampal neurons by introducing various intracellular agents. Voltage-clamp studies were carried out using acutely dissociated hippocampal neurons from adult guinea pigs. Synaptic events were examined using intracellular recordings in the slice preparation. They found that the anion fluoride could affect calcium conductance by an intracellular action. When anions other than fluoride were used for intracellular recordings, the voltage-dependent calcium current inactivated slowly and showed persistent activation at membrane potentials between -40 and -10 mV. In contrast, when fluoride was present intracellularly, the inactivation kinetics of the calcium current were accelerated and the persistent component of the current was largely suppressed. Intracellular recordings in the hippocampal slice showed that when electrodes contained cesium, QX 314, and fluoride, the spiking and nonlinear responses of the neuronal membrane to depolarization were blocked. FULL STUDY