guanosine 5'-[$\alpha\beta$ -imido]diphosphate) is required to be bound in the site concomitantly with aluminofluoride to get activation. This simple test should have been done by Stadel & Crooke.

The fact that non-hydrolysable analogues of GTP compete successfully with AlF_4^- in G-proteins is also expected in the phosphate-analogue model, since the binding of these analogues is irreversible while aluminofluoride binding is reversible: an irreversible reaction always overrides a reversible one.

The phosphate analogue model of aluminofluoride is most likely valid for all the G-proteins involved in transduction of membrane receptor signals, all of them having very analogous nucleotide-binding sites [4,5]. Beyond the field of G-proteins, this model could recently account for the interesting effects of aluminofluoride or beryllifluoride on tubulin [8], on phosphatases [9], 'P' type cation transport ATPase [10,11] and H^+ -ATPase [12]. It seems to be of general interest for the study of all types of enzymes which have a nucleotide cofactor or simply a phosphate group binding site.

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Fluoride interaction with G-proteins

Chabre and his colleagues have proposed that an ionic complex of AlF_4^- activates G-proteins by interacting with GDP at the nucleotide binding site [1]. They suggest that the AlF_4^- complex mimics the terminal γ -phosphate of GTP and thereby promotes activation. Current data do not rule out alternative possibilities that AlF_3 is the active species [2] or that Al^{3+} and F^- could bind to separate sites on G-proteins. Chabre clearly explains the basics of the 'ternary complex model' that can successfully explain the binding properties of G-protein-coupled receptors with agonists [3]. Chabre has interpreted this

model in terms of rhodopsin-transducin interactions. The nuances of the model, as applied to receptor-adenylate cyclase coupling, suggested to us a different interpretation of fluoride interaction with G-proteins of adenylate cyclase [4]. Two points are central to our hypothesis: the stoichiometry of the components of the signal transduction pathway and the reversible nature of their interactions.

Chabre suggests that the amount of the stimulatory G-protein of adenylate cyclase (G_s) in membranes may be in excess to its receptor (R_s) and that agonist (A) occupancy of R_s drives the formation of a ternary complex, $A-R_s-G_s$, to completion. The percentage of R_s induced to form a high-affinity, nucleotide-sensitive state was found to be dependent on the intrinsic activity of A. In the presence of full agonists only 80–90% of R_s formed a high-affinity ternary complex, the remaining 10–20% of R_s were measurable in a low-affinity binding state [3]. This observation suggested that G_s may be limiting or at least not present in a large excess to R_s . Quantification of G_s by cholera toxin-catalysed [^{32}P]ADP-ribosylation provided biochemical evidence in support of a near-equal stoichiometry [5,6]. On the second point, fluoride activation of G_s in detergent solution or in reconstituted lipid vesicles is reversible under appropriate conditions [7,8]. However, the activation of G_s in membranes by fluoride leads to the irreversible activation of the adenylate cyclase catalytic unit (C). Exogenous nucleotide is not needed for this activation, possibly due to the tight binding of GDP to G_s in its basal state, and the stimulation of the cyclase activity appears to be due to the stable association of the α -subunit of G_s with C [9]. Thus, stimulation of adenylate cyclase activity by fluoride is apparently analogous to activation by nonhydrolysable GTP analogues. Calculations based on the specific activity of purified C [10]

Fig. 1. Inhibition of [^3H]hydroxybenzylisoproterenol ([^3H]HBI) binding by GDP to frog erythrocyte membranes pre-treated with and without NaF

Frog erythrocyte membranes were prepared and incubated in the presence (○) or absence (●) of 20 mM-NaF for 20 min at 30 °C as described previously [4]. Binding assays were performed as described previously [4]. Each data point represents the mean $\pm$ S.E.M. of duplicate determinations from three experiments

estimate that C is present in membranes in amounts comparable to R_s . We attempted to take advantage of the irreversible nature of fluoride-dependent stimulation of adenylate cyclase activity to examine the mechanism of fluoride action. Membranes, preincubated for 20 min with fluoride concentrations that maximally stimulated adenylate cyclase activity, were used to initiate binding assays, which included fluoride during the incubations [4]. Since the fluoride-stimulated adenylate cyclase activity was not reversed by agonist or agonist plus guanine nucleotide under the conditions of the binding assay, we were indeed surprised to observe no effect of the fluoride pretreatment on the extent of agonist high-affinity binding nor on the sensitivity of the A- R_s high-affinity binding state to a GTP analogue. These results were difficult to reconcile with Chabre's model if G_s activation was due to AlF_4^- mimicking the GTP γ -phosphate in the nucleotide binding site. Our data were consistent with those of Brandt & Ross [8]. They investigated the effects of Al^{3+} and F^- on catecholamine-stimulated GTPase, GDP release and GTP binding using pure R_s and G_s reconstituted in lipid vesicles. Al^{3+} - F^- had little effect on these assays and moreover, agonist plus guanine nucleotide had no effect on deactivation of G_s in vesicles preincubated with Al^{3+} - F^- . These observations are in contrast to the inhibition by Al^{3+} - F^- of rhodopsin-stimulated GTPase and nucleotide binding of transducin [11].

Chabre suggested an experiment to test further the mechanism of Al^{3+} - F^- interaction with G_s during radioligand binding assays. As can be seen in Fig. 1, GDP was more potent in inhibiting the binding of agonist [3H]-hydroxybenzylisoproterenol to β -adrenergic receptors in membranes pretreated with fluoride than in control membranes preincubated in buffer alone. The observation that the fluoride effect was selective for GDP compared with GTP analogues (see Fig. 4 of [4]) or GMP (results not shown) is consistent with the model proposed by Bigay *et al.* [1]. However, since the triphosphate form of guanine nucleotides is not required for the modulation of R_s affinity for A, this experiment does not precisely

define the site of fluoride action. In a study contemporary to our own, Ransnas & Insel [12] have shown by immunochemical techniques that G_s is present in S49 lymphoma cell membranes in a large stoichiometric excess to R_s . It is uncertain if all or only a portion of the measured G_s is available for coupling to R_s [12]. These data indicate that [^{32}P]ADP-ribosylation may underestimate the amount of G_s in membrane preparations. In light of these new results, alternative interpretations of our original data are possible. Further experimentation will be required to elucidate the mechanism by which Al^{3+} - F^- activates G-proteins.

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