

approaches the rate of the slow phase of desensitization. These results indicate that the rapid phase of desensitization could have an absolute dependence on phosphorylation. We have been unable to test this hypothesis because we have not been able to prepare a completely dephosphorylated preparation of the nAChR.

The nAChR is phosphorylated by three different protein kinases: cyclic AMP-dependent protein kinase¹⁸, protein kinase C^{19,20} and a tyrosine-specific-protein kinase¹⁰. Cyclic AMP-dependent phosphorylation of the γ and δ subunits of the purified nAChR increases the rate of the rapid phase of desensitization¹⁶. In addition, studies with intact muscle^{21,22} and cultured muscle cells^{23,24} have shown that cAMP analogues and forskolin (both activators of cAMP-dependent protein kinase) and phorbol esters (activators of protein kinase C) increase the rate of desensitization of the receptor. These results, taken together with those presented here, suggest that phosphorylation of the receptor by each of the three protein kinase systems, presumably in response to three different first messenger pathways, regulates the rate of desensitization of the nAChR. The first messengers that regulate phosphorylation of the nAChR by protein kinase C and the tyrosine-specific protein kinase are not known. However it has been reported recently that calcitonin gene-related peptide (CGRP), a neuropeptide that is present at the neuromuscular junction, regulates phosphorylation of the nAChR²⁵ and the rate of desensitization of the nAChR in myotubes²⁶ by the activation of cAMP-dependent protein kinase. Desensitization is a form of short-term regulation of synaptic efficacy in the second-to-minute time range²⁷⁻²⁹. Protein phosphorylation appears to be an important way of modulating this process.

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The G protein-gated atrial K⁺ channel is stimulated by three distinct G_iα-subunits

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The guanine nucleotide-binding protein, G_i, which inhibits adenylyl cyclase, has recently been shown to have three subtypes of the α-subunit, termed G_iα-1, G_iα-2 and G_iα-3. They share 87-94% amino-acid sequence homology¹⁻¹⁰ and so are difficult to separate from one another. Among other functions^{11,12-14}, purified preparations activate K⁺ channels¹⁵⁻²¹ but there is confusion over which of the subtypes activates the muscarinic K⁺ channels of the atrial muscle of the heart: G_iα-3, also termed G_k¹⁵, has been shown to activate this channel but it is not clear whether G_iα-1²² does²¹ or does not^{23,24}. To clarify this problem, we expressed the subtypes separately in *Escherichia coli* to eliminate contamination by other subtypes and tested the recombinant α-chains on atrial muscarinic K⁺ channels. Although we anticipated that only G_iα-3 would have G_k activity, to our surprise all three recombinant subtypes were active, from which we deduce that the G_i subtypes are multifunctional.

We modified the Gα complementary DNAs by site-directed mutagenesis, if necessary, to retain a unique C'CATGG *Nco*I site at the start of their open reading frames and inserted the

cDNAs into the *Bam*HI site of the cloning cassette of the pT7-7 expression vector²⁵. This construction led to the expression of recombinant Gα fusion polypeptides that differed from their natural counterparts by an N-terminal extension of nine amino acids. The recombinants constituted 5-10% of the total cell protein²⁶ of which 5% was recovered in a GTPγS-activated form after partial purification by DEAE-cellulose chromatography²⁶.

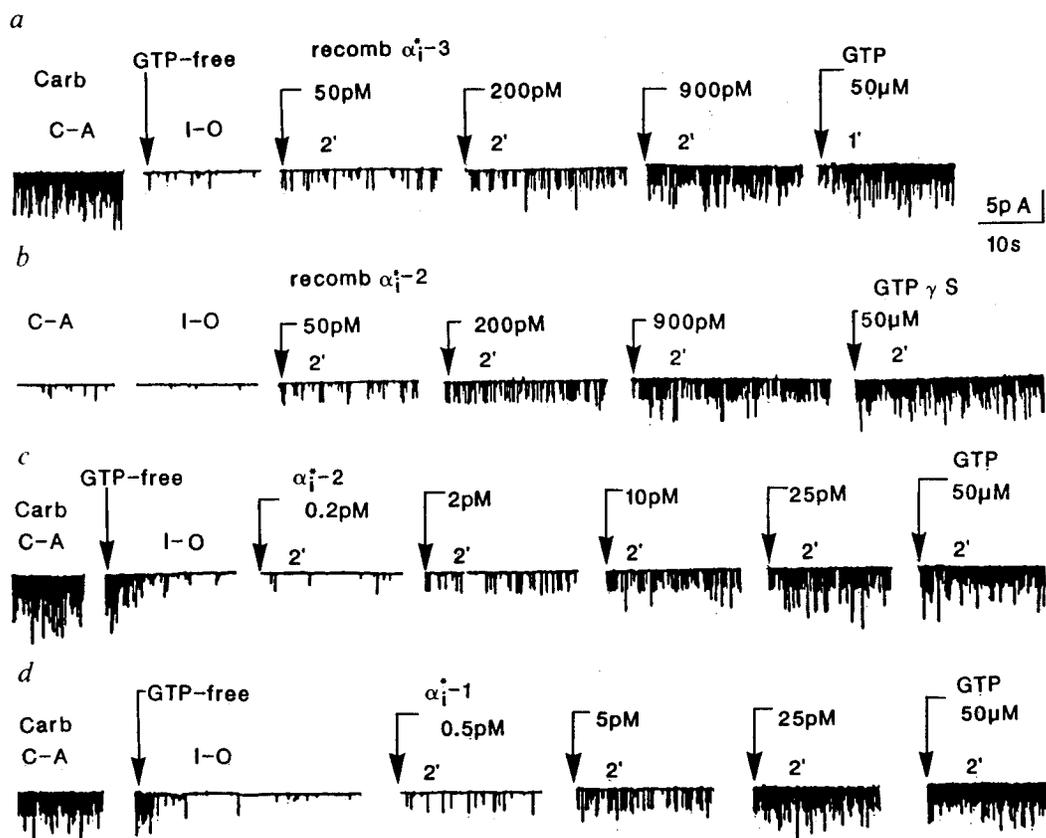
We found that the GTPγS-activated recombinant forms of G_iα-3, G_iα-1 and G_iα-2 stimulated specific atrial K⁺ channels (Figs 1 and 2). The conductances and mean open times were identical to those of atrial K⁺ channels stimulated by carbachol plus GTP or GTPγS (Table 1). Maximal activation by any one of the recombinant G_iα-subunits was not additive with stimulation by agonist plus GTP or GTPγS (Fig. 1). The stimulation was concentration dependent: concentration-response curves were constructed to compare intrinsic potencies. As for activated native human erythrocyte G_iα-3¹⁵, the stimulatory effects of the GTPγS-activated recombinant G_iα-subunits could not be washed out and were essentially irreversible (data not shown). Hence, the effectiveness of added G_iα-subunits depended on concentration and time. Currents produced at each concentration were compared from about 120 s after subunit addition for the next 20 s (Fig. 2). For normalization, the maximal patch currents were measured with 100 nM carbachol in the pipette and 50-100 μM GTP in the bath, or after addition of 50-100 μM GTPγS to the bath. Under these conditions we found the three forms of recombinant G_iα-subunits were equipotent (Fig. 2) with one difference: the frequency of success with recombinant G_iα-1 seemed lower. Our impression is that failures result when access to the patch is restricted due to partial sealing over. This seems to affect recombinant G_iα-1 more than recombinants G_iα-2 and G_iα-3.

The following tests proved that the effects observed with each of the recombinant G_iα-subunits were intrinsic properties of

Fig. 1 Effects of GTP γ S-activated G β α -2 and G β α -3 recombinant subunits (recomb α_i^* -3 (a) and recomb α_i^* -2 (b)), and of natural GTP γ S-activated G β α -subunits (α_i^* -2, c; α_i^* -1, d) on single atrial muscarinic K $^+$ -channel currents of inside-out membrane patches excised from single atrial cells of adult guinea pigs¹⁵. The effects of recombinant α_i^* -1 are shown in Fig. 2.

Methods. Recombinant α -subunits were induced in *E. coli*, extracted, activated with GTP γ S and purified partially, as previously described²⁶. Natural G β α -subunits were prepared¹⁶ from bovine brain G β α -1 (E. Padrell & R. Iyengar, manuscript in preparation) and human erythrocyte G β α -2²⁸. The proteins were diluted in bathing solution¹⁵ and added to inside-out membrane patches as described¹⁶. Single channel K $^+$ currents were recorded and analysed as described^{15,16,27}. In a, c

and d, carbachol (Carb) was in the pipette at 10 $^{-7}$ M. The effects are compared to those of GTP (in the presence of carbachol) or GTP γ S (in the absence of agonist, b) in each experiment. The results were unaffected by the presence or absence of carbachol in the pipette.



the expressed molecules. (1) Boiling before addition to the bath abolished the activity of each recombinant G β α -subunit ($n = 6$). (2) Partially-purified proteins from bacteria transfected with the expression plasmid having antisense cDNAs or partially purified, GTP γ S-activated recombinant G β α -subunit which stimulates adenylyl cyclase and dihydropyridine-sensitive Ca $^{2+}$ channels²⁷, had no effect ($n = 18$). (3) Threshold concentrations of GTP γ S for activation of atrial muscarinic K $^+$ channels in inside-out membrane patches with 100 nM carbachol in the pipette were 10–100 nM, at least 10 times the maximum GTP γ S added with saturating concentrations (1 nM) of any of the GTP γ S-activated recombinant G β α -subunits. Prior addition of

100 μ M GDP β S, which blocked carbachol-mediated effects of 10 μ M GTP, did not interfere with the effects of the GTP γ S-activated recombinant G β α -subunits.

Prompted by the results with the G β α -1 and G β α -2 recombinant subunits, we purified natural G β α -1 from bovine brain (E. Padrell and R. Iyengar, unpublished results) and G β α -2 from human erythrocytes²⁸, prepared their GTP γ S-activated forms¹⁶ and confirmed that both had potent G β α activity (Fig. 1). Half-maximal effects were obtained with approximately 10–30 pM of G β α -1 and G β α -2 (not shown), values not significantly different from those previously obtained with human erythrocyte G β α -3^{15,16,19}.

Figure 2 shows that the concentrations of recombinant G β α -subunits needed for half-maximal effects were approximately 500 pM, about 25 times higher than the half-maximal concentrations for the native G β α -subunits. The maximal currents produced, however, were similar. Another bacterial fusion polypeptide, a recombinant G β α -subunit, stimulates adenylyl cyclase and dihydropyridine-sensitive Ca $^{2+}$ channels with less potency than the native protein²⁸. Perhaps incomplete or aberrant post-translation processing is responsible for the lower affinity of the recombinant polypeptides for their effectors.

These experiments showed that native G β α -1, G β α -2 and G β α -3 reproduced the molecular behaviour of the natural atrial G β α protein (Table 1). Now, as before²⁹, it is unclear why bovine brain GTP γ S-activated G β α repeatedly failed to activate the atrial muscarinic K $^+$ channel^{23,24}. Positive results would be expected regardless of the exact identity (G β α -1, G β α -2 or G β α -3) of the pertussis toxin substrate used in those experiments.

The demonstration that all three G β α -subunits are isoforms with respect to K $^+$ channel regulation leads us to conclude that one or more of these G proteins must be multifunctional. Previous work has shown that another G protein, G β , is multifunctional, and can stimulate both adenylyl cyclase and dihy-

Table 1 Comparison of single atrial K $^+$ -channel currents activated by carbachol plus GTP, GTP γ S and the three types of GTP γ S-activated recombinant G β α -subunits

| Conditions | Mean open time (ms) | Conductance (pS) | <i>n</i> |
|-----------------------------------|---------------------|------------------|----------|
| Agonist + GTP | 1.8 \pm 0.6 | 40 \pm 2 | 18 |
| GTP γ S | 1.8 \pm 0.8 | 40 \pm 1 | 8 |
| Recombinant G β α -1 | 1.6 \pm 0.5 | 40 \pm 1 | 7 |
| Recombinant G β α -2 | 1.7 \pm 0.7 | 40 \pm 2 | 6 |
| Recombinant G β α -3 | 1.8 \pm 0.5 | 39 \pm 2 | 8 |

Data were from inside-out membranes patches excised from adult guinea pig atrial cells. Solutions were symmetrical isotonic K $^+$ solutions¹⁵. When present, carbachol was at 10 $^{-7}$ M; GTP at 10–100 μ M; GTP γ S at 50–100 μ M; and recombinant G β α -subunits, at 0.2–1 nM. In some experiments AMP-P(NH)P 200 μ M was present. Single-channel currents were analysed with a laboratory computer (PDP 11/73, Digital Equipment Corp.) as described previously^{15,16,27}. Slope conductances were measured between –40 and –120 mV in half the experiments and chord conductances were measured at –40 mV in the remainder. *n* is the number of experiments and values are expressed as means \pm s.e.m.

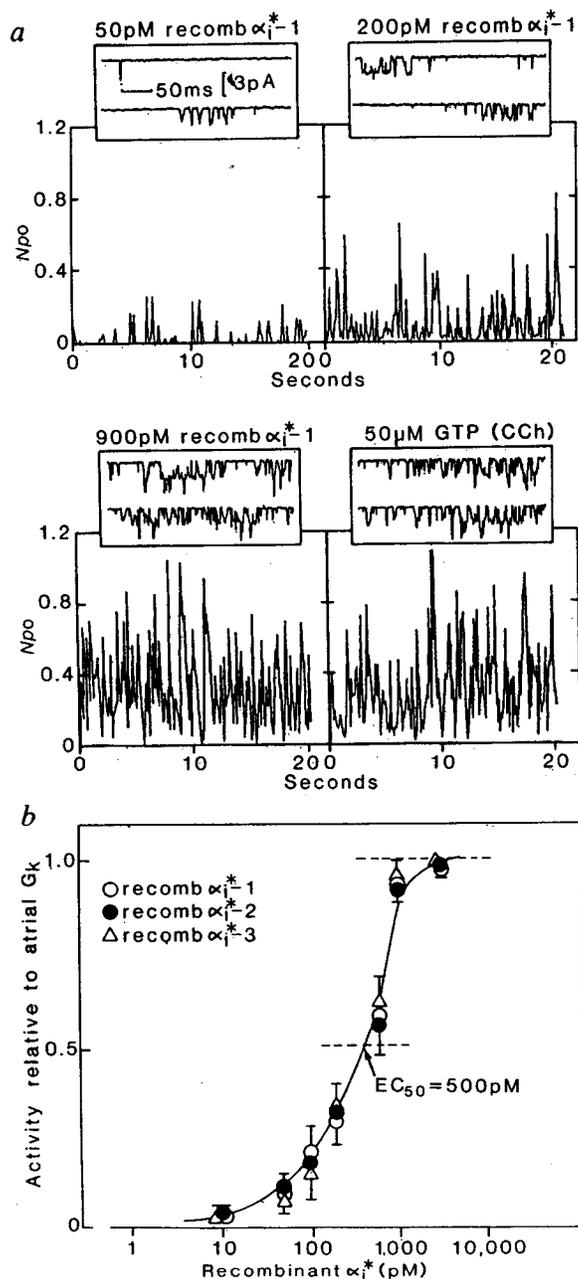


Fig. 2 Concentration-response relationships for recombinant α^* -subunits. In *a*, recombinant $G_i\alpha^*-1$ (recomb α^*-1) was added at 50, 200 and 900 pM. Insets are the single channel currents at -80 mV in symmetrical K^+ solutions^{15,16}. Filter frequency at -3 dB was 1 kHz and sampling rate was 5 kHz. The proportion of open time was measured every 100 ms and plotted as Np_0 (N is the number of channels; p_0 , the probability of the channel being open at that potential) versus time, to give the diaries shown in the main panels. Np_0 was averaged over 200 such segments beginning in each case 120 s after addition of recombinant α^*-1 and the mean values were 0.028, top left, 0.0903 top right and 0.319 (bottom left). The addition of 100 μ M GTP to the bathing solution elicited no response beyond that obtained with 900 pM recombinant $G_i\alpha^*-1$ (bottom right mean value was 0.320). In *b*, the mean values obtained with recombinant $G_i\alpha^*-1$, $G_i\alpha^*-2$ and $G_i\alpha^*-3$, were normalized to maximum Np_0 values obtained either by adding 50–100 μ M GTP when carbachol at 10^{-7} M was in the pipette or by adding 50–100 μ M GTP γ S when carbachol was absent, and were plotted against subunit concentrations. Data points were mean \pm s.e.m. from 4 to 6 patches. The concentration-response relationships obtained for each of the three types of subunits were superimposable, had a Hill coefficient greater than one and reached 50% of maximum at 500 pM (EC_{50}) of added recombinant $G_i\alpha^*$ -subunits.

dropyridine-sensitive Ca^{2+} channels^{26,27,30}, indicating that many G proteins may be multifunctional. In the world of signal transduction, a G protein does not simply regulate one effector; it executes a programme of responses.

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Conservation of the D-mannose-adhesion protein among type 1 fimbriated members of the family Enterobacteriaceae

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A variety of genera and species of the family Enterobacteriaceae bear surface fimbriae that enable them to bind to D-mannose residues on eukaryotic cells^{1–3}. Until recently, it was thought that the D-mannose binding site was located in the major structural subunit (FimA), of relative molecular mass (M_r) 17,000 (17 K), of these organelles in *Escherichia coli*^{4,5}. New evidence indicates that this binding site resides instead in a minor protein M_r 28–31 K (FimH) located at the tips and at long intervals along the length of the fimbriae^{6–12}, and is reminiscent of the minor tip adhesion proteins of pyelonephritis-associated pili (Pap) and S fimbriae^{13,14}. In contrast to the antigenic heterogeneity of the major FimA subunit, the antigenic structure of FimH is conserved among different strains of *E. coli*^{10,11}. Here, we report an even broader conservation of this minor adhesion protein extending to other genera and species of type 1 fimbriated Enterobacteriaceae. Our results may have implications for the development of broadly protective vaccines against Gram-negative bacillary infections in animals and perhaps in man.

Type 1 fimbriae were isolated from two random clinical isolates each of *E. coli*, *Klebsiella pneumoniae*, *Serratia marcescens*

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