20 during sperm maturation. It is unlikely that changes in the density of PH-20 are responsible for the change in its mobility, since diffusion is fastest on acrosome-reacted sperm where PH-20 is the most dense (*23*). Rather, the present results indicate that restriction of lateral mobility of a membrane protein can be mediated exclusively by interactions of its ectodomain.

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19. Because of the nature of the FRAP technique, we are measuring the diffusion of labeled membrane proteins. We do not know if the presence of the Fab' label alters the intrinsic diffusion coefficient of the protein. Nevertheless, our conclusion that the diffusion of PI-anchored proteins can be limited by interactions with the ectodomain remains valid.
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24. Testicular sperm were enriched to between 80% and 90% by density separation through an isotonic 52% Percoll gradient before ^{125}I labeling. Epididymal sperm were isolated from the distal tubules of the cauda epididymis (*25*). After surface iodination, >90% of the epididymal sperm were induced to undergo the acrosome reaction by the addition of the calcium ionophore A23187 (2 μg/ml) (*23*). Because the sperm were in each case iodinated while they were acrosome-intact, we observed only the population of PH-20 on the plasma membrane and not the additional internal pool that becomes exposed on the surface after the exocytotic acrosome reaction (*23*).
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The α Subunit of the GTP Binding Protein Activates Muscarinic Potassium Channels of the Atrium

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It has been debated whether the potassium channel of the atrium is activated by the α subunit or by the βγ subunits of guanine nucleotide binding (G) proteins, which dissociate on activation with guanosine triphosphate (GTP). Therefore, the channel-activating effectiveness of these subunits on isolated guinea pig atrial cells was tested. The activated α_K subunit from human erythrocytes activated the channel in subpicomolar concentrations. The βγ dimer from bovine brain activated the channel in nanomolar concentrations. These results support the view that, physiologically, the α subunit activates the channel.

WHEN ACETYLCHOLINE (ACh) OR adenosine activates the sarcolemmal potassium channel (K^+_{ACh} channel) of mammalian or chicken atria, receptor occupation is coupled to channel opening via guanine nucleotide binding (G) proteins (*1–4*). These G proteins are composed of three subunits, α, β, and γ, and it is thought that binding of the agonist to the receptor leads to binding of guanosine triphosphate (GTP) to the α subunit and that activated α (α*) in turn dissociates from the βγ dimer (*5, 6*). After dissociation of the αβγ trimer, the channel is opened (*1*), but whether opening results from interaction with the α* subunit or with the βγ dimer is a matter of controversy. Logothetis *et al.* unexpectedly failed to demonstrate activation of the K^+_{ACh} channel (from chicken) with the α subunit, but they were able to show K^+ channel opening with nanomolar concentrations of the βγ dimer (*7*). Brown and co-workers reported activation of mammalian K^+_{ACh} channels by picomolar concentrations of the α subunit (α^*_K) from human erythrocytes (*6, 8, 9*). Whether K^+_{ACh} channels are activated by α or βγ, or both, is important for understanding receptor-effector coupling.

We therefore studied activation of K^+_{ACh} channels in atrial myocytes from adult guinea pigs (*10*). The isolated cells were bathed in a medium containing 140 m*M* KCl, 1 m*M* K_2EGTA, and 3 m*M* magnesium adenosine triphosphate (ATP) that was adjusted to *p*H 7.4 by 5 m*M* Hepes-tris buffer. Single channel currents were recorded at room temperature (22° to 23°C) by gigaohm-seal patch clamping (*11*). The patch pipettes were filled with the bath medium but without MgATP.

In a first series of control experiments, we activated the K^+_{ACh} channels in cell-attached patches by adding 0.1 μ*M* carbachol, the muscarinic agonist, to the pipette. From 12 experiments, we evaluated an open channel conductance of 41 ± 5 pS (slope conductance between −40 and −100 mV) and a mean lifetime of the open state of 2.9 ± 0.4 ms (mean ± SEM). For the second series of control experiments, we excised the patch from the cell. In the resulting inside-out configuration, channel activity gradually disappeared within 1 to 5 min, but it reappeared when we added 100 μ*M* GTPγS [guanosine-5'-O-(1-thiotriphosphate)] to the bath (*1, 7, 8*). The GTPγS-activated channel (n = 10) had a conductance of 37 ± 3 pS and a mean channel open time of 2.1 ± 0.2 ms. Thus, the K^+_{ACh} currents activated by GTPγS are indistinguishable from those resulting from receptor occupation by carbachol (*1*). Because GTPγS activated the K^+_{ACh} channels very reliably, we used GTPγS activation as a criterion to decide if the protein was ineffective or if the channel was lost, for example, by vesicle formation in the patch. The GTPγS concentrations below 0.1 μ*M* did not activate the K^+_{ACh} channel; this result seems to exclude the possibility that picomolar amounts of GTPγS, contaminating the preactivated α^*_K, are responsible for activation of endogenous G proteins and K^+_{ACh} channels.

The activating effect of 0.4 p*M* α^*_K is shown by the on-line registration of Fig. 1A. The computer trace (Fig. 1C) demonstrates that the currents have a unitary amplitude of about 4 pA (patch potential −80 mV). In six patches treated with 4 p*M* α^*_K, we evaluated amplitude at potentials between −40 and −100 mV, plotted them in a current-voltage (I-V) curve and obtained from the slope a conductance of 41 ± 4 pS. The mean channel open time evaluated from histograms (Fig. 1E) was 2.2 ± 0.2 ms. Conductance and open time are indistinguishable from their counterparts measured under activation by carbachol or by GTPγS or from the values reported in the literature (*1, 4, 7, 9*). Thus the α^*_K subunit of the G protein effectively opens the K^+_{ACh} channel at picomolar concentrations.

This conclusion confirms the results of Brown and colleagues (*9*). We have seen this

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result in 22 preparations to which the α_K^* subunit was applied. Activation by α_K^* started at a threshold concentration of 0.4 p*M*; in nine experiments we observed five positive results and four failures. When we applied 4 p*M* α_K^*, none of the seven trials failed. Usually the activity of the K^+_{ACh} channel reached a steady value within 2 to 5 min; the channel openness (open probability times number of K^+_{ACh} channels in the patch) increased from less than 0.1% to about 10%, mostly by reduction of the long closures. Higher α_K^* concentrations activated the K^+_{ACh} channel to a greater extent. Because the number of channels in the patch was unknown, we could not determine a dose-response curve.

We also could activate the K^+_{ACh} channel by using the $\beta\gamma$ dimer when it was prepared as in (7) (Fig. 1). The on-line record in Fig. 1B shows the disappearance of channel activity after excision of the patch. Afterward, moderate channel activity was induced by adding 184 μM CHAPS {3-[(3-cholamidopropyl)-dimethyl-ammoniol] 1-propane sulfonate} to the bath, which is used to stabilize the $\beta\gamma$ dimer (7). Finally, in the presence of 184 μM CHAPS, the addition of 30 n*M* $\beta\gamma$ dimer resulted in a pronounced increase in channel activity. Channel activation became steady within less than 1 min and was reversible upon washout. In 12 experiments, the $\beta\gamma$ dimer activated the K^+_{ACh} channel at concentrations of 10 n*M* or more (no failures). At $\beta\gamma$ dimer concentrations of 5 n*M* (seven trials), we were successful three times and failed four times in channel activation; $\beta\gamma$ dimer concentrations of 1 n*M*, 0.1 n*M*, or less were ineffective. Thus, 5 n*M* is considered the threshold concentration.

The current through channels induced by $\beta\gamma$ dimer cannot be distinguished from the one after α_K^* activation (compare Fig. 1, C and D). Channel activity induced by 15 n*M* $\beta\gamma$ dimer had a conductance of 43 ± 6 pS and a mean open time of 2.2 ± 0.5 ms (compare Fig. 1, E and F). These data are indistinguishable from those in which the K^+_{ACh} channel was activated by carbachol, GTPγS, or the α_K^* subunit. They reproduce the results of Clapham and colleagues (7), but with about tenfold larger concentrations [threshold of 5 n*M* and not 0.2 n*M* as in (*12*)]. Our results suggest that the K^+_{ACh} channel is activated by both α_K^* and $\beta\gamma$ preparations.

Could K^+_{ACh} channel activation result from the presence of the detergent CHAPS? Our results show that application of $\beta\gamma$ dimer plus CHAPS is more effective in activating K^+_{ACh} channels than is CHAPS alone. After exposure to CHAPS for 5 min, subsequent addition of 30 n*M* $\beta\gamma$ dimer increased the channel openness by a factor of 5 (n = 3) (Fig. 1B). In four out of nine trials 184 μM CHAPS did not activate the K^+_{ACh} channels. Also, boiling the aliquot that contained 184 μM CHAPS plus 25 n*M* $\beta\gamma$ dimer for 10 min abolished the channel-activating properties (n = 3). When K^+_{ACh} channels were activated by CHAPS alone, they had a conductance of 39 ± 4 pS, which is indistinguishable from the conductance induced by the $\beta\gamma$ dimer or the α subunit. But K^+_{ACh} channels activated by CHAPS were peculiar in that they had a mean open time distribution with two time constants (6 ms and 0.6 ms, respectively, n = 5).

Thus, we can activate the K^+_{ACh} channel with the preactivated α_K^* subunit from human erythrocytes. We suggest physiological activation of K^+_{ACh} channels by $\beta\gamma$ dimers to be unlikely because it requires a concentration about 10,000-fold higher than activation by the α^* subunit. Although activation of K^+_{ACh} channels by the $\beta\gamma$ dimer preparation seems to be real, it is not easy to interpret. It has been attributed to contamination with α_K^* (*8*), which it is not possible to resolve if the relative effectiveness is 1:10,000. Alternatively, the observed $\beta\gamma$ effects could result from channel activation by CHAPS if this detergent interacts with the channel more efficiently when it is bound and translocated by the $\beta\gamma$ dimer as a carrier. Experiments with CHAPS-free $\beta\gamma$ may clarify this point. From a teleological standpoint, present knowledge favors the idea that it is the α^* subunit that activates the K^+_{ACh} channel; the agonist specificity is easily explained by the large number of diverse (specific) α subunits but difficult to imagine in terms of identical $\beta\gamma$ dimers that are released by several G proteins.

Note added in proof: Activation of the muscarinic ACh channel by the α_K has recently been confirmed (*13*).

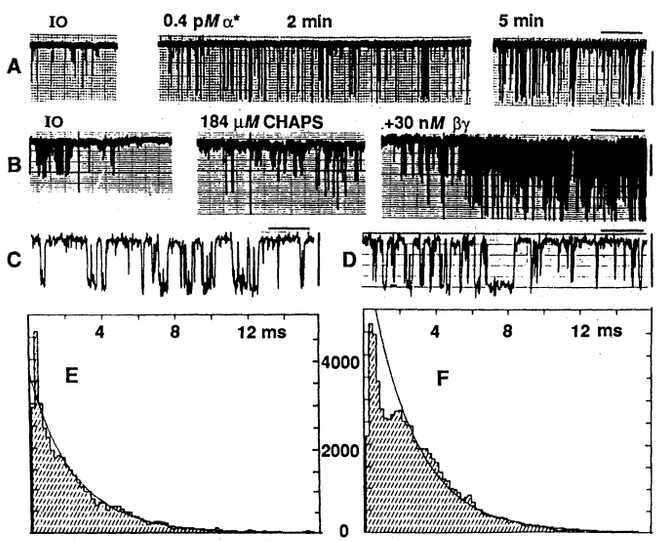

Fig. 1. Muscarinic K^+ channel activation by the components of the G protein. Inside-out (IO) patch recordings from adult guinea pig atrial myocytes. (**A**) On-line pen recording showing induction of single channel currents by 0.4 p*M* α_K^*. Preactivated α_K^* was diluted from a 2 μM stock solution to 0.8 p*M*, which was added as a 500-μl aliquot to a 500-μl chamber. The bath was mixed with the pipette. The minutes mark the time elapsed after addition of α_K^*. Calibration bars, 20 s and 4 pA. The single-channel currents appear to vary in amplitude because the limited frequency response of the pen recorder attenuates the short events more than the long ones. (**B**) On-line pen recording showing the disappearance of channel activity after excision of the patch (IO), induction of activity by CHAPS, and enhancement of channel activity when 30 n*M* $\beta\gamma$ dimer was added in the constant presence of CHAPS. The $\beta\gamma$ dimer was obtained as a 3.8 μM stock solution, diluted with the CHAPS-containing bath solution to 60 n*M*, and added as 500-μl aliquot to the 500-μl bath. The patch contains at least two channels. Calibration bars, 20 s and 4 pA. (**C**) Currents through single K^+_{ACh} channels activated by 4 p*M* α_K^*. Record from computer playback (PDP 11-73). The data were filtered at 2 kHz and sampled at 200-μs per point. Calibration bars, 40 ms and 4 pA. (**D**) Currents through single K^+_{ACh} channels activated by 30 n*M* $\beta\gamma$ dimer with 184 μM CHAPS. Computer playback as in (C). (**E**) Distribution of the single-channel open time duration for K^+_{ACh} channels induced by 4 p*M* α_K^*. The fitting line follows a single exponential with a decay time constant of 2.4 ms. (**F**) Distribution of the single-channel open time duration for K^+_{ACh} channels induced by 30 n*M* $\beta\gamma$ with 184 μM CHAPS, fitted with a time constant of 25 ms.

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