

The Nature and Origin of Spontaneous Noise in G Protein-gated Ion Channels

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ABSTRACT Arrival of agonist is generally thought to initiate the signal transduction process in G protein-receptor coupled systems. However, the muscarinic atrial K⁺ (K⁺[ACh]) channel opens spontaneously in the absence of applied agonist, giving a noisy appearance to the current records. We investigated the nature and origin of the noise by measuring single channel currents in cell-attached or excised, inside-out membrane patches. Guanosine triphosphate (GTP) produced identical single channel currents in a concentration- and Mg²⁺-dependent manner in the presence or absence of carbachol, but the requirements for GTP were greater in the absence of agonist. Hence the agonist-independent currents appeared to be produced by an endogenous G protein, G_k. This prediction was confirmed when an affinity-purified, sequence-specific G_i-3 α antibody or pertussis toxin (PTX) blocked the agonist-independent currents. Candidate endogenous agonists were ruled out by the lack of effect of their corresponding antagonists. Thus agonist-independent currents had the same nature as agonist-dependent K⁺[ACh] currents and seemed to originate in the same way. We have developed a hypothesis in which agonist-free, empty receptors prime G_k with GTP and G_k activates atrial K⁺ [ACh] channels producing basal currents or noise. Agonist-independent activation by G proteins of effectors including ion channels appears to be a common occurrence.

INTRODUCTION

Membrane noise influences the learning performance of neurons (Buhmann and Schulten, 1987), and the mechanisms by which noise is produced may be important to our understanding of the nervous system. In postsynaptic cells membrane noise arises from the release of neurotransmitter, which produces stochastic openings and closings of ligand-gated ion channels. However, this may not be the only mechanism by which noise is produced. In postsynaptic atrial muscle cells acetylcholine activates a muscarinic K⁺ channel (K⁺[ACh]) via a G protein referred to as G_k (Breitwieser and Szabo, 1985), which is probably G_i-3 or G_i-2 (Codina et al., 1987; Yatani et al., 1988). But atrial K⁺[ACh] channels have been reported to open spontaneously in the absence of applied agonist (Soejima and Noma, 1984; Logothetis et al., 1987; Yatani et al., 1987a), making the basal current records noisy as a result. Similar spontaneous

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currents have been observed for G_o -gated K^+ channels in hippocampal neurons (VanDongen et al., 1988). However, the phenomenon is not limited to excitable cells; spontaneous membrane noise also occurs in G protein-gated ion channels in nonexcitable epithelial cells (Cantiello et al., 1989; Light et al., 1989). Since nothing is known about the nature or origin of the spontaneous openings, we investigated the basal noise of atrial $K^+[ACh]$ channels by measuring single channel $K^+[ACh]$ currents in cell-attached (C-A) membrane patches and examined the mechanism more closely in excised, inside-out (I-O) patches. We found that agonist-dependent and independent $K^+[ACh]$ currents were identical. We propose that G_k activated by GTP produced the agonist-independent currents, and agonist-free, empty receptors seemed necessary for the activation of G_k by GTP.

METHODS

Single atrial myocytes were isolated from guinea pig hearts by enzymatic dissociation following the procedure described previously (Brown et al., 1984). After digestion, the heart was washed with KB medium (Isenberg and Klöckner, 1982), kept at room temperature, and used for electrophysiological study within 8 h. Single channel currents were recorded using the patch-clamp method with a List EPC 7 amplifier (Hamill et al., 1981). The patch pipettes had tip resistances of 5–10 M Ω and were filled with an isotonic K^+ solution composed of (in mM): 140 KCl, 2 MgCl₂, 5 EGTA, and 5 HEPES (pH 7.4 with Tris base). Cells were placed in a recording chamber (0.5 ml) containing the same solution. All experiments were performed at room temperature (20–22°C). All nucleotides were obtained from Boehringer-Mannheim GmbH (Mannheim, Germany). Other agents were from Sigma Chemical Co. (St. Louis, MO).

Continuous records of channel activity were stored on videocassette tape for subsequent analysis. Single channel currents were analyzed using a laboratory computer (PDP 11/73) by methods previously described (Lux and Brown, 1984). Briefly, the records were low-pass filtered at 2 kHz (–3 dB) and digitized at 5–10 kHz. Slow time base records were displayed directly on a strip chart recorder having a frequency response (–3 dB) of 100 Hz. Transitions were idealized by half-maximum amplitude detection. Amplitude histograms were fit with Gaussian functions and open times were fit to exponential probability density functions. Parameter estimates for all data fits were obtained using a maximum likelihood estimator.

All test agents were applied by the concentration-clamp method, which allows solution changes within 10 ms (Akaike et al., 1986; Yatani and Brown, 1989). The tip of the patch pipette with the excised membrane was inserted into a polyethylene tube, which served as a host chamber, through a ~1-mm-diam circular hole. The lower end of this tube was exposed directly to external solutions by moving up and down the stage upon which drug-containing chambers were located and then rotating the appropriate chambers into position. The solutions were changed by applying suction (–15 cmHg) to the upper end of the host chamber. The suction was controlled by an electromagnetic valve which was switched on for a desired duration by a stimulator (type S44; Grass Instrument Co., Quincy, MA). Mechanical or switching transients did not interfere with the currents.

To measure concentration-dependent effects of guanine nucleotides, we averaged idealized single channel currents. This was done by measuring the proportion of open time P for N channels in the patch pipette, together called NP . NP was integrated for variable periods between 200 and 400 ms and the average value was determined. Accumulated NP values were also plotted as cumulative NP . Steady-state concentration–response relationships were measured as NP determined 1 min after the jumps were made for a period of 20 s and normalized to the maximum NP value obtained with GTP γ S (100 μ M) at the end of each experiment (see Fig. 1, *A* and *B*). The ratio of average NP at each concentration of guanine nucleotides to maximum NP was plotted against concentration. The concentration–response data were fit to

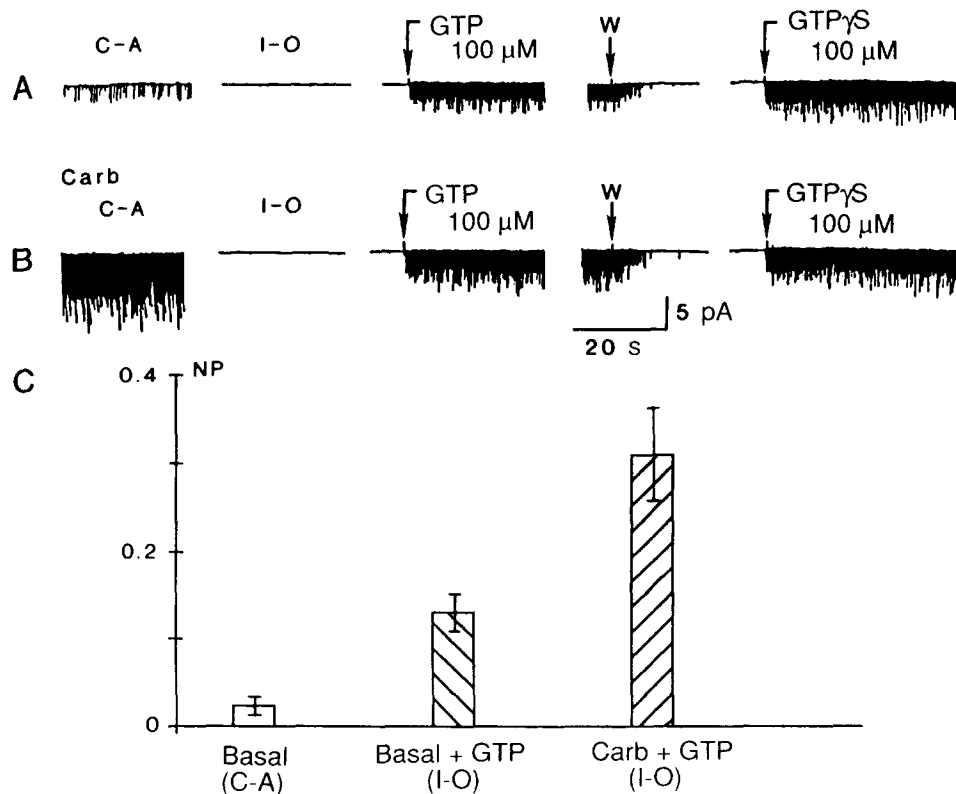


FIGURE 1. Spontaneous and agonist-dependent carb-activated single channel currents in C-A and agonist-dependent and -independent single channel currents in excised I-O patches. Representative C-A currents in the absence (A) and presence (B) of carb (10 μM) in the pipette. In A, patch solution contained 10 μM atropine, 100 μM theophylline, and 10 μM prazosin. 2 mM AMP-PNP was present in the bath solution in A and B. The holding potential was 80 mV negative from the resting potential, assumed to be zero in the isotonic K⁺ solution in C-A recording, and -80 mV in the I-O configuration. Concentration steps of guanine nucleotides were applied at arrows. Concentration steps back to zero guanine nucleotides were at the arrows below W (for washout). Currents were continuous and gaps between records were 30–60 s. C shows NP for the basal activity in C-A configuration (left-hand bar) and NP's measured in I-O mode in the absence (middle bar) and presence (right-hand bar) of carb (10 μM) plus GTP (100 μM). Values were mean ± SEM of 7, 10, and 11 experiments for basal (C-A), basal + GTP (I-O), and carb + GTP (I-O), respectively.

single occupancy Langmuir absorption isotherms. The time course of activation or deactivation was determined by averaging NP and fitting the curve to an exponential function.

RESULTS

Nature of Agonist-dependent, Agonist-independent, and Spontaneously Arising Single Channel K⁺[ACh] Currents

Single channel K⁺[ACh]-like currents were present in C-A patches of individual atrial myocytes in the absence of agonist (Fig. 1A), confirming earlier observations

(Soejima and Noma, 1984; Yatani et al., 1987a; Logothetis et al., 1987). These currents had a unitary conductance and mean open time (Fig. 2A) identical to the currents produced by muscarinic agonists such as carbachol (carb) (Fig. 1B, 2B, Table I), but the opening probability (P_o) was greatly reduced (Fig. 1C). We refer to these currents as spontaneous or basal currents because they occur under normal cellular conditions. Identical single channel currents were recorded from excised I-O patches in the absence of agonist, provided GTP and Mg^{2+} were added to the bath

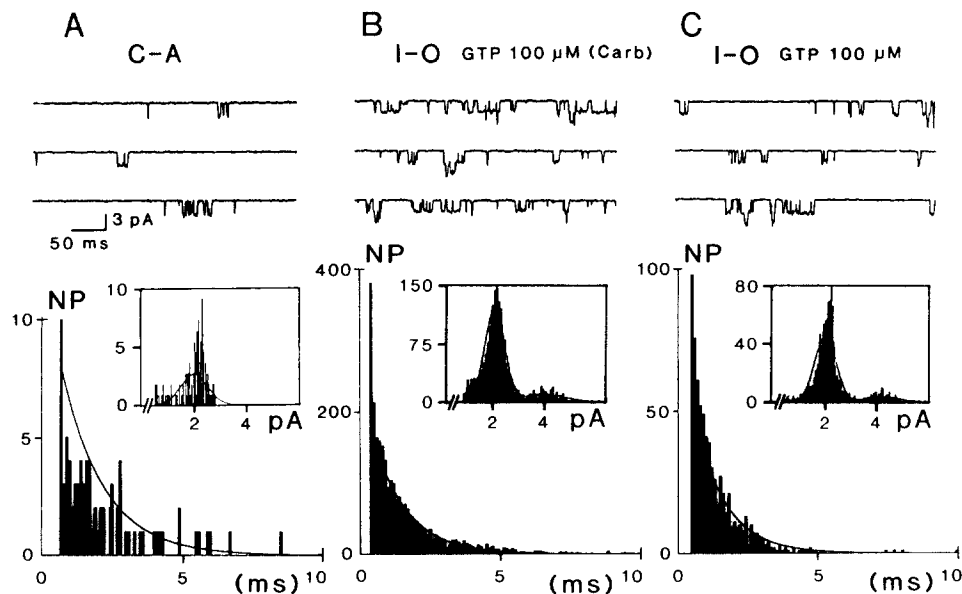


FIGURE 2. Single channel properties of spontaneous or basal C-A currents (A), carb (10 μ M)-activated I-O currents with 100 μ M GTP (B), and agonist-independent I-O currents with 100 μ M GTP (C). The currents were low-pass filtered at 1 kHz and sampled at 5 kHz. Calibrations in A apply to all modes. Frequency histograms of open times and amplitudes are shown below. The histograms were constructed from 20 s of recording and analyzed at 10 kHz. The mean open times were 1.0, 1.0, and 0.9 ms for A, B, and C, respectively. The solid lines were fit to τ 's of 1.1, 1.2, and 1.1 ms, respectively. The continuous curves on the amplitude histograms were Gaussian distributions and the parameters were fitted by a maximum likelihood estimator. Mean amplitudes were 2.0 pA (A), 2.0 and 4.0 pA (B), and 2.0 and 4.2 pA (C). The single channel conductances between -40 and -100 mV were identical in the three conditions (~ 40 pS). The larger amplitudes arose from two simultaneous openings.

solution (Fig. 1, Table I). We refer to these as agonist-independent currents. The absolute requirement for GTP and Mg^{2+} was also true for agonist-dependent currents (Kurachi et al., 1986a; Brown and Yatani, 1990), and the effects of GTP were reversible in both cases (Fig. 1). In subsequent experiments we probed the roles of each element using the agents indicated in the figure. Just as for agonist-activated currents, GDP or GDP β S (100 μ M) blocked agonist-independent currents. All three currents are therefore assumed to arise from K^+ [ACh] channels. We assumed a

minimum system of three elements, muscarinic acetylcholine receptor, endogenous G_k , and the $K^+[ACh]$ channel (Fig. 3), and the fact that GTP repeatedly reconstituted either response showed that all three elements remained in the patch.

Production of Agonist by Excised Membrane Patches Is Unlikely to Produce Agonist-independent Currents

If the agonist-independent currents arose from an endogenous agonist, then the agonist should have been continuously produced by either the membrane patch or nerve terminals avulsed with it. We tested these possibilities by adding to the pipette solution: (a) the muscarinic antagonist atropine or scopolamine at 10 μM ($n = 8$); (b) atropine combined with the purinergic receptor antagonist theophylline at 100 μM ($n = 8$); (c) atropine, theophylline, and an α_1 -adrenergic blocker prazosin at 10 μM ($n = 4$); and by adding to the bath solution the inhibitory ATP substrate AMP-PNP at 2 mM ($n = 4$). In all cases basal openings were present in C-A patches (Fig. 1A) and responded as the controls did to changes in bath GTP concentrations after patch excision. These concentrations of atropine and theophylline completely blocked the

TABLE I
Properties of Single Channel Atrial K^+ Currents in the Absence and Presence of Agonist

Recording conditions	Mean amplitude	Mean open time	n^*
	pA	ms	
Basal (C-A mode)	2.0 ± 0.1	1.0 ± 0.3	5
GTP (I-O mode)	2.1 ± 0.1	1.3 ± 0.4	5
GTP + Carb (I-O mode)	2.1 ± 0.1	1.4 ± 0.2	7

* n = experimental number.

Mean values are obtained from 300–600 events per experiment for basal C-A mode and from 1,000–3,000 events per experiment for I-O mode in each experiment. Data are means \pm SD. GTP concentration was 100 μM and carb was 10 μM .

effects of ACh and adenosine (Kurachi et al., 1986b), respectively. Arachidonic acid metabolites which activate $K^+[ACh]$ currents would seem to be ruled out since arachidonic acid was ineffective in I-O patches (Kim et al., 1989; Kurachi et al., 1989a). Furthermore, cardiac myocytes do not have lipoxygenase pathways (Hohl and Rösen, 1987) and the cyclooxygenase pathway did not project to $K^+[ACh]$ channels (Yatani et al., 1990a). GTP, when applied extracellularly, did not activate $K^+[ACh]$ currents, so any leakage of bath GTP into the patch pipette would not be a factor. While these experiments can never completely exclude the possibility of an endogenous agonist, they do rule out candidates known to activate $K^+[ACh]$ channels. With this proviso we will assume that the receptors are free of agonist or empty (Costa and Herz, 1989).

Are G Proteins Mediating Agonist-independent Activation?

The next issue was whether endogenous G proteins activated agonist-free $K^+[ACh]$ channels. If so, were they the same as the G_k that coupled M_2 receptors to these channels? We examined this with affinity-purified antibodies to COOH-terminal

decapeptides specific for G_{α} or G_{γ} - 3α (Simonds et al., 1989). The anti- G_{γ} - 3α antibody was applied at dilutions of 10^3 – 10^4 . The anti- G_{γ} - 3α antibody completely blocked agonist-independent currents at 2.4 nM ($n = 6$) and carb-activated currents ($n = 8$) at concentrations of 10 nM (Fig. 4). The antibody produced its effects on agonist-independent and carb-activated currents by reducing opening frequency without changing the unitary current amplitude (not shown). The block was faster for agonist-independent currents and in both cases the block could not be relieved by washing for 5 min or longer. The block of both agonist-independent and carb-activated currents was relieved by GTP γ S (100 μ M). The rate at which GTP γ S acted, however, was far slower than control (cf. Figs. 1 and 4) and resembled the slowed rate of GTP γ S activation after PTX (Fig. 5). A possible explanation is that GTP γ S first bound to the holo-G protein and then promoted antibody dissociation before activating K^+ [ACh] channels. The antibody effects were probably specific because: (a)

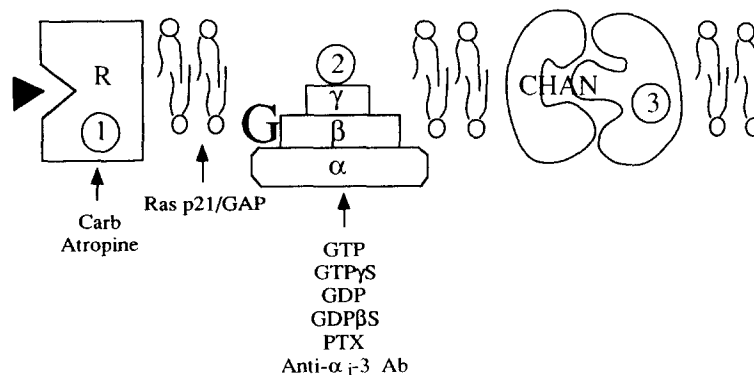


FIGURE 3. Minimum vectorial pathway for information flow: muscarinic cholinergic receptor, G_k , and K^+ [ACh] channel. *R*, receptor; *G*, signal-transducing G protein and its α -, β -, and γ -subunits (referred to in text as G_k); *Chan*, K^+ [ACh] channel; *PTX*, pertussis toxin; *Anti- α -3 Ab*, sequence-specific antibody to G_{α_3} ; *ras p21/GAP*, combination of the *ras* p21 protein and *ras* p21 GTPase activating protein (GAP).

the G_{α} antibody, which does not immunoreact with G_{α} (Simonds et al., 1989), had no effect on either agonist-independent or carb-activated currents ($n = 10$) at concentrations of 10 nM; and (b) preimmune IgG at concentrations of 200 nM was ineffective ($n = 5$). The results support an earlier conclusion that G_{γ} - 3α may be G_k (Codina et al., 1987; Yatani et al., 1988) and point to the same G protein coupling agonist-free and agonist-occupied receptors to K^+ [ACh] channels.

Uncoupling or Inactivating Empty Receptors

If empty receptors were responsible for agonist-free currents, then uncoupling them from endogenous G_k might abolish the single channel K^+ [ACh] currents. For G_{γ} proteins, PTX can achieve this without changing nucleotide hydrolysis or release of GDP (Sunyer et al., 1989). This condition was satisfied using activated PTX and NAD^+ (Kurachi et al., 1986b; Yatani et al., 1987b) and the prediction was confirmed.

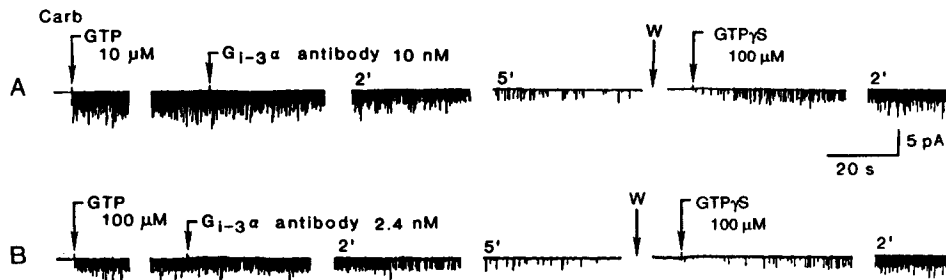


FIGURE 4. Identifying the endogenous G protein that activates basal K⁺[ACh] currents: effects of a sequence-specific G_{i-3α} antibody on carb-activated (A) and agonist-independent (B) K⁺[ACh] currents in excised I-O patches. Test agents and washes were applied by concentration jumps at arrows. The numbers above each segment are the time (′ = minutes) elapsed after the step change in concentration. Gaps were 30–60 s unless indicated otherwise and wash periods were 2 min. Holding potential was -80 mV. Concentrations of G_{i-3α} antibody were assumed to have a molecular weight of 180,000.

PTX at the concentrations we used completely blocked agonist-dependent and independent currents (Fig. 5). The latter were, however, always blocked more quickly. In both cases the block was due to a reduced frequency of opening since the single channel currents had unchanged amplitudes and open times (not shown). The block of currents could not be overcome by GTP even at concentrations of 1.0 mM. However, GTPγS was effective although the rate at which GTPγS activated the fully blocked single channel currents was slowed as it was after exposure to the G_{i-3α} antibody (cf. Figs. 1, 4, and 5). Thus, coupling of receptor to G protein may be essential for agonist-free K⁺[ACh] currents under the condition that ADP ribosylation by PTX had only an uncoupling effect (Van Dop et al., 1984; Gilman, 1987; Sullivan et al., 1987; Sunyer et al., 1989).

Rate of Agonist-independent Activation Depends upon GTP Concentration

To test the role of receptors further, we examined an important concept in signal transduction by G proteins; namely, that the agonist-bound receptor increases the

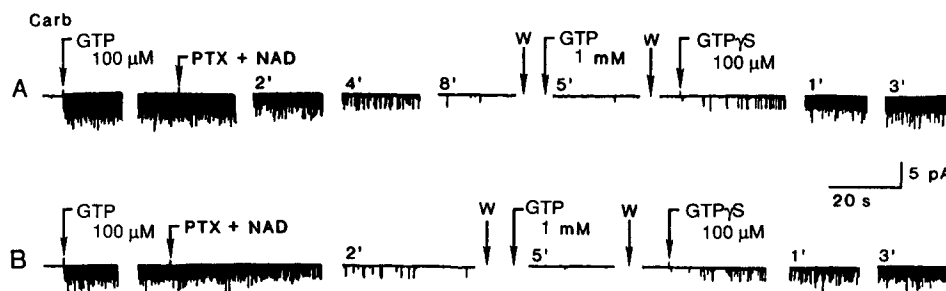


FIGURE 5. Uncoupling the endogenous G protein by preactivated PTX plus NAD⁺. Single channel K⁺[ACh] currents were activated by GTP in the presence (A) and absence of agonist (B). Concentrations of activated PTX and NAD⁺ were 0.4 μg/ml and 0.4 mM, respectively. Recording conditions were identical to those in Fig. 4.

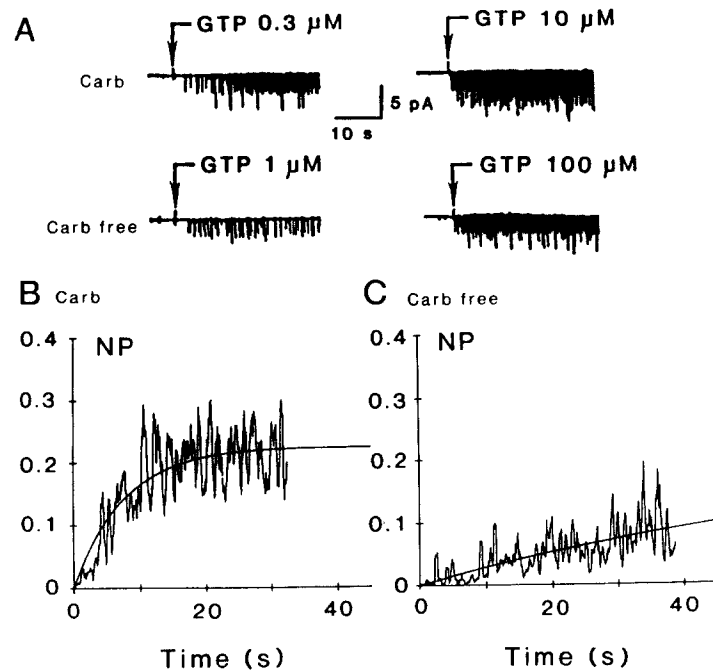


FIGURE 6. Effects of different concentrations of GTP on rate of activation of agonist-dependent and -independent single channel $K^+[ACh]$ currents (A). Carb (10 μM) was present in the pipette solution in top traces and absent in bottom traces. (B) Time course of NP averaged every 200 ms after a concentration jump of GTP (0.3 μM) in the presence of carb (10 μM). The delay was 100 ms and the activation $T_{1/2}$ was 7.5 s. (C) Time course NP for a concentration jump of GTP (0.3 μM) in the absence of carb. The delay was 3 s and the $T_{1/2}$ was 41 s.

TABLE II
Concentration-dependent Rates of Activation of $K^+[ACh]$ Currents by GTP in the Absence and Presence of Agonist

GTP	Agonist-free $T_{1/2}^*$	n^i	Carb $T_{1/2}$	n^i
μM	s		s	
0.1	ND ^b	—	30 ± 10	5
0.3	35 ± 7	3	8 ± 3	6
1	18 ± 4	4	2 ± 0.4	4
5	ND	—	1.1 ± 0.3	13
10	1.1 ± 0.4	4	1.0 ± 0.4	10
100	1.0 ± 0.3	9	1.0 ± 0.3	8
1,000	0.8 ± 0.1	5	0.7 ± 0.2	4

* $T_{1/2}$ = half activation time (see Fig. 6, B and C).

ⁱ n = number of experiments. Data are mean \pm SD.

^bND = not determined. Carb concentration was 10 μM .

rate at which GDP leaves the $G\alpha$ subunit (Cassel and Selinger, 1976; Gilman, 1987). In the absence of agonist the α -subunit is mostly GDP-bound because the GTPase rate is $\sim 4 \text{ min}^{-1}$ and the off rate for GDP is $\sim 0.4 \text{ min}^{-1}$ (Gilman, 1987). In the case of purified receptor-independent G protein, unless the GDP was removed, the rate of binding of a guanine nucleotide such as GTP γ S to $G\alpha$ was found to be independent

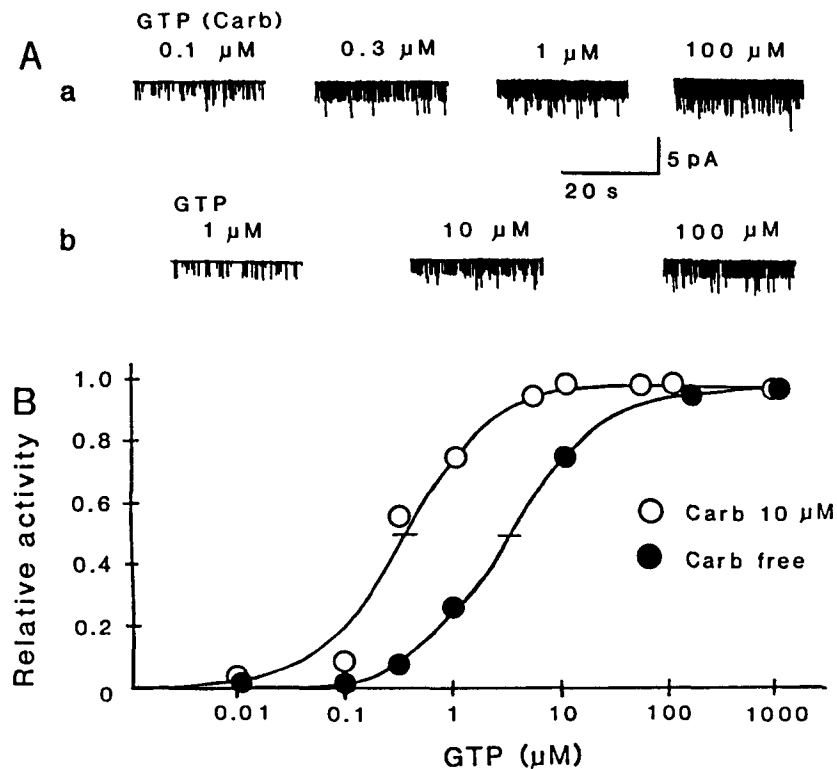


FIGURE 7. (A) Steady-state GTP concentration-dependent activation of single channel $K^+[ACh]$ currents. Carb (10 μM) was present in *a* and absent in *b*. (B) Steady-state GTP concentration-response curves for agonist-dependent (\circ) and agonist-independent (\bullet) single channel $K^+[ACh]$ currents. The extent of activation at different concentrations was compared at 60 s after concentration jumps of GTP. NP at each concentration of GTP was normalized to the NP obtained by GTP γ S (100 μM) at the end of each experiment. In the absence of carb, the maximum NP was 0.5 ± 0.2 ($n = 20$) of the maximum obtained in the presence of agonist. Data points were mean values of 3–13 independent patches. Curves were fit by a one to one Langmuir adsorption isotherm. EC_{50} 's were 0.3 and 3 μM for agonist-dependent and -independent activation, respectively.

of its concentration (Ferguson et al., 1986). Assuming that the G protein behaved similarly in situ, if the empty receptors have no effect on the coupling of endogenous G proteins, then for reasonably rapid changes of GTP concentration the rate of activation by GTP should be of zero order with respect to concentration. However, if

empty receptors activated G_x , which then gated $K^+[ACh]$ channels as the PTX results suggested, another test would be whether GTP activated basal currents at rates that were concentration dependent.

With the concentration clamp solutions are exchanged at the membrane within 10 ms after a delay of 50 ms (Akaike et al., 1986; Okabe et al., 1989; Yatani and Brown, 1989). To test this, the GTP concentration was jumped from zero to different values (Fig. 6). Single channel $K^+[ACh]$ currents began to increase after a further delay and reached a new, steady level over the next several seconds (Fig. 6*A*). The rate and extent of activation were concentration dependent in the presence and absence of agonist. After jumping back to zero, GTP currents ceased within 20 s (Okabe et al., 1989). Repeated trials gave similar responses. Only $K^+[ACh]$ currents were increased; ATP-sensitive K^+ currents (Noma, 1983) and voltage-dependent, inwardly rectifying K^+ currents (Sakmann and Trube, 1984), which are also present in these membrane patches (Yatani et al., 1990*a*), were unaffected. Because single channel currents are stochastic, we quantified the changes by averaging the single channel currents and fitting the average current with an exponential function after allowing for a delay (Fig. 6, *B* and *C*). The half-time, $T_{1/2}$, was strongly dependent on the concentration of GTP at lower concentrations and became concentration independent at higher concentrations in both the agonist-free and agonist cases (Table II). For the basal case the $T_{1/2}$'s were shifted to higher concentrations.

It was not only the rates of activation that were agonist and GTP dependent; steady-state $K^+[ACh]$ currents also responded to jumps in GTP, depending on whether or not agonist was present. Half-maximal activation of the steady-state concentration–response curves in agonist-free and activated states were 3.0 and 0.3 μM , respectively (Fig. 7). The maximum agonist-free currents were 0.5 ± 0.2 times the maximum agonist-activated currents ($n = 20$). GDP shifted the steady-state GTP concentration–current curve to higher concentrations and slowed the activation rates (not shown). Both effects occurred competitively with GTP. An intracellular GTP/GDP ratio of 10 would account for the level of basal single channel $K^+[ACh]$ currents observed in the C-A configuration (Soejima and Noma, 1984; Logothetis et al., 1987; Yatani et al., 1987*a*).

DISCUSSION

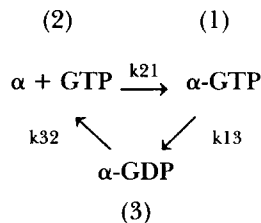
We interpret our results with a hypothesis in which agonist-free, empty receptors activate a PTX-sensitive endogenous G protein, possibly G_{i-3} , which in turn activates $K^+[ACh]$ channels. We assume that the interaction between the G protein and the $K^+[ACh]$ channel is direct, but this has not been shown because the channel protein has not yet been purified (Brown and Birnbaumer, 1988). For the purified G protein, G_s , and the purified dihydropyridine receptor Ca^{2+} channel, a direct effect has been shown (Hamilton et al., 1990). Our hypothesis is based on the findings that: (*a*) basal noise currents are identical to agonist-activated currents; (*b*) candidate endogenous agonists have been excluded; (*c*) specific $G_{i-3\alpha}$ antibodies block agonist-independent

activation; (d) PTX also blocks activation and is reported to have no effects on GTP-ase activity, binding of GTP, or release of GDP from $G_i\alpha$ (Van Dop et al., 1984; Gilman, 1987; Sullivan et al., 1987; Sunyer et al., 1989); and (e) *ras* p21 in combination with the GTPase activating protein (GAP) blocked basal atrial $K^+[ACh]$ currents (Yatani et al., 1990b). However, *ras* p21-GAP did not interact with either G_i-3 or the $K^+[ACh]$ channel, so that the block was upstream of the G protein between it and the receptor (Yatani et al., 1990b). These points are summarized in Fig. 3.

Spontaneous, agonist-independent single channel $K^+[ACh]$ currents were identical to currents produced by muscarinic agonists (Soejima and Noma, 1984; Logothetis et al., 1987; Yatani et al., 1987a), purinergic agonists (Kurachi et al., 1986b), GTP γ S or Gpp(NH)P (Kurachi et al., 1986b; Logothetis et al., 1987; Yatani et al., 1987a), preactivated exogenous $G_i\alpha$ s (Codina et al., 1987; Logothetis et al., 1988), or arachidonic acid metabolites (Kim et al., 1989; Kurachi et al., 1989a,b). As a result, we cannot determine which receptors were involved in basal or agonist-free activation, or indeed whether there is any specificity at all. Nor do we know what the range of basal activity may be except that the GTP/GDP ratio will be an important factor (Breitwieser and Szabo, 1988). Another factor regulating basal activity may be levels of the dimeric $\beta\gamma$ -subunits of G proteins since dimeric $\beta\gamma$ inhibited agonist-occupied and -independent $K^+[ACh]$ channels (Okabe et al., 1990).

Our hypothesis seems to apply to spontaneous openings reported for G_o -gated K^+ channels in hippocampal neurons (VanDongen et al., 1988). If this is so, membrane noise in postsynaptic cells need not arise only from stochastic release of neurotransmitter; it may also arise from G proteins activated by empty receptors. Furthermore, agonist-independent G protein activation is probably not restricted to the nervous system, and may also apply to Na^+ or Cl^- channels in epithelial cells (Cantiello, 1989; Light et al., 1989). Moreover, ionic channels may not be the only G protein effectors activated in the absence of agonist. Reconstitution experiments using β -adrenoreceptors, G_s , and adenylyl cyclase may be consistent with our hypothesis although they shed no light on whether adenylyl cyclase is noisy in native membranes. In one set of results agonist-free receptors clearly activated GTPase activity (Cerione et al., 1984), while in another they did not (May et al., 1985). Unfortunately, the reconstitution experiments were complicated by the use of thiol-activated receptors. The reconstitution experiments also did not establish whether adenylyl cyclase operates in the same way in the presence or absence of agonist.

What are some of the consequences when ion channels are G protein effectors for empty receptors? First, the basal noise will contribute to setting the membrane potential and in this way provide a broader range over which membrane potential can be regulated. Second, agonists can produce a more rapid response on a system that is idling rather than fully stopped. To illustrate this point we have developed a minimum three-state Markov chain (Kemeny and Small, 1976; Cox and Miller, 1980) consisting of receptor, G_k , and $K^+[ACh]$ channel (Fig. 3). A similar model was treated by Breitwieser and Szabo (1988). The states were connected by transition rate constants for the catalytic rate of $G_i\alpha$ -GTP, the release of bound GDP and the binding of GTP.



In the model, receptor promotes k_{32} and $\alpha\text{-GTP}$ reacts immediately with $\text{K}^+[\text{ACh}]$ channels. The probability (P) with which any state was occupied was given by:

$$dP(t)/dt = P(t)Q$$

where Q was a matrix with elements q_{ij} that corresponded to the transition rate constants from state i to state j . The analytical solution had the general form:

$$P(t) = B[A_1 \exp(\lambda_1 t) + A_2 \exp(\lambda_2 t) + A_3]C$$

where P was a scalar and was the probability of being in any one of three states, B was a 1×3 row vector containing the initial occupancy probabilities, C was a 3×1 column vector giving the measured state, A 's were the eigenvectors, and λ 's were the eigenvalues for the Q matrix. This equation was fit to step responses such as those in Fig. 6 using a maximum likelihood estimator and from the two rate constants a $T_{1/2}$ was calculated. The $T_{1/2}$'s were similar to the experimentally determined values shown in Table II. Initial values for k_{21} , k_{13} , and k_{32} were 9.4, 0.10, and 0.007 s^{-1} , respectively, and initial steady-state occupancies $P(2)$, $P(1)$, and $P(3)$ were 0.00068, 0.064, and 0.935, respectively. By giving k_{32} a nonzero value, agonist-free receptor primed the system and the nonzero occupancy of state 2 produced a faster response to agonist.

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