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29. Daily production calculated from the time-of-day corrected photosynthetic characteristics was compared to the carbon incorporated during a 6-hour incubation (1200 to 1800) extrapolated to 24 hours. Although we have assumed that photosynthesis was light-saturated throughout the day, since α and P_m^B covaried (*22*), the diel patterns of photosynthesis would be similar at both photosynthesis-limiting and saturating irradiances. Incident irradiances during the austral summer are typically >200 μE m^{-2} $sec.^{-1}$ at midnight and 1200 to 1600 μE m^{-2} sec^{-1} at noon. If we assume a diffuse attenuation coefficient of 0.15 m^{-1} [(*5, 8*); R. B. Rivkin, unpublished data] and an I_k of 39 μE m^{-2} sec^{-1}, photosynthesis would be light-saturated in the upper 10-m depths at midnight and 15- to 25-m depths during the day. Similar irradiances saturated photosynthesis for phytoplankton assemblages collected both under the sea ice and in open water at the ice edge (*19*).
30. During time-course incubations under natural photoperiods and at photosynthesis-limiting and saturating irradiances in December and January, carbon uptake was 2.6 to 4.2 times greater for samples incubated for 24 hours than during either 6-hour (0600 to 1200) or 12-hour (0600 to 1800) incubations extrapolated to 24 hours. Daily production was 950 to 1200 mg of carbon per square meter per day and 225 to 356 mg of carbon per square meter per day for 24-hour and 6- or 12-hour incubations, respectively {a euphotic zone depth of 0.1% incident irradiance was assumed [(*5, 11*); R. B. Rivkin, unpublished data]}.
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A G Protein Directly Regulates Mammalian Cardiac Calcium Channels

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A possible direct effect of guanine nucleotide binding (G) proteins on calcium channels was examined in membrane patches excised from guinea pig cardiac myocytes and bovine cardiac sarcolemmal vesicles incorporated into planar lipid bilayers. The guanosine triphosphate analog, GTPγS, prolonged the survival of excised calcium channels independently of the presence of adenosine 3′,5′-monophosphate (cAMP), adenosine triphosphate, cAMP-activated protein kinase, and the protein kinase C activator tetradecanoyl phorbol acetate. A specific G protein, activated G_s, or its α subunit, purified from the plasma membranes of human erythrocytes, prolonged the survival of excised channels and stimulated the activity of incorporated channels. Thus, in addition to regulating calcium channels indirectly through activation of cytoplasmic kinases, G proteins can regulate calcium channels directly. Since they also directly regulate a subset of potassium channels, G proteins are now known to directly gate two classes of membrane ion channels.

GUANINE NUCLEOTIDE BINDING (G) proteins couple a variety of plasma membrane receptors to voltage-dependent calcium channels, and, for cardiac β-adrenoreceptors, the mechanism is indirect and involves cytoplasmic second messengers (*1*). G proteins can also directly couple membrane receptors to ion channels independently of cytoplasmic mediators. The G protein G_k directly activates subsets of potassium channels that normally are activated through muscarinic cholinergic (*2*) or somatostatin receptors (*3*), or both. In this report, we tested whether G proteins also modulate Ca^{2+} channels directly.

Single Ca^{2+} channel activity was recorded in guinea pig ventricular myocytes, first in the cell-attached configuration and then, after excision, in the inside-out configuration. We compared voltage-dependent activation of Ca^{2+} channels under different experimental conditions (Table 1), using a protocol in which the membrane patches were subjected to depolarizing test pulses of 200-msec duration at 0.5 Hz, before and after excision. Confirming a well-known finding, activatable responses survived only briefly after excision from nonstimulated cells (*4*). However, survival was enhanced by stimulation of channel activity during the cell-attached mode by the β adrenoreceptor agonist isoproterenol (ISO). For quantification, we both counted the number of test pulses during which activity was observable after excision (Table 1) and evaluated the current conducted by the membrane patches during the depolarizing pulses (Fig. 1). The results depend on the number (N) of channels in the patch and on their probability of opening during the test pulse. To provide a record of the entire experiment we measured, before and after patch excision, the proportion of open time (P) for the N channels in the patch (NP) for each pulse (Fig. 1, row 2). NP values were also summed to give cumulative activities in each patch (Fig. 1, row 3). To normalize for patch to patch variations in channel number, the cumulative activities summed over comparable number of traces (usually 30) before (cum NP_{CA}) and after (cum NP_{IO}) patch excision were compared (Table 1). All the results were from high-threshold Ca^{2+} channels; no low-threshold, dihydropyridine-insensitive Ca^{2+} channels (*5*) were observed, and polarizing the membrane to −90 mV from the usual values between −40 and −60 mV before applying test pulses failed to unmask any that might have been inactivated.

A small number of events was recorded in nonactivated control cells; the test potentials used to detect Ca^{2+} channel activity in cell-attached patches produced average opening probabilities (P_o's) of 0.01 to 0.05 at room temperature. The very brief survival after excision provided few data for comparison (Table 1). Consequently, we tried to increase NP with ISO. When ISO at $10^{-5}M$ was present only in the patch pipette ($n = 7$), P_o seemed greater, and prolonged openings, although still less than 1% of the events (*6*), seemed more frequent. Activity was not recorded without ISO in the pipette, so the significance of the changes was unclear (*7*). We then added ISO at $10^{-6}M$ to the bath; this was followed after several seconds by a large increase in NP ($n = 3$), confirming previous reports (*8*). Subsequently, we incubated the myocytes with ISO ($10^{-6}M$). The increase in NP was due to both increased frequency of opening and prolongation of events (*8*) and was associated with an increase in survival (Table 1). After excision, the mean open times were unchanged but the amplitudes could change, reflecting a change in driving force (*9*). When guanosine 5′-*O*-(3-thio)triphosphate (GTPγS) (100 μM) was present in the bath, survival was enhanced considerably (Fig. 1 and Table 1). In fact, activity was

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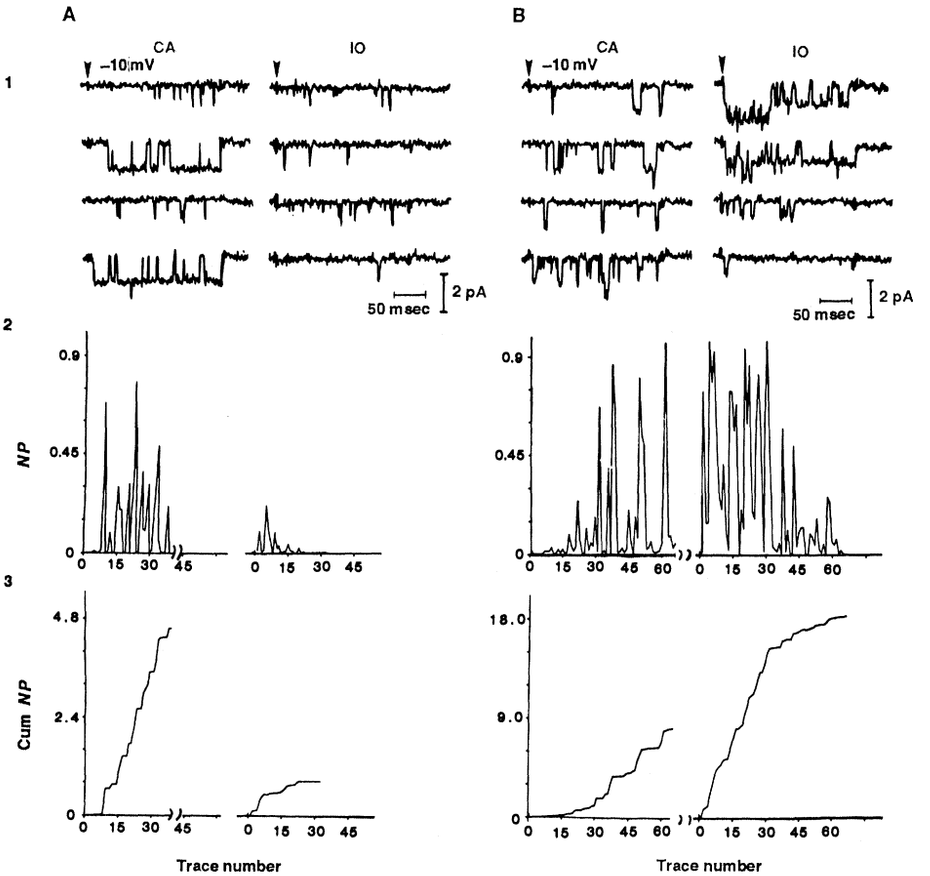

Fig. 1. Effects of GTPγS on Ca^{2+} channels in inside-out membrane patches excised from ISO-treated cardiac myocytes. Single ventricular cells were obtained from guinea pig hearts by enzymatic dispersion (*2*). Single channel recordings were made from cell-attached (CA) and inside-out (IO) patches (*21, 22*). The records in row 1 were filtered at 1 kHz. Experiments were done in (**A**) the absence and (**B**) the presence of GTPγS. In both cases, ISO was present in the bath ($10^{-6}M$) and in the patch pipette ($10^{-5}M$). (Row 1) Four rows of single-channel current traces representative of the total number plotted in row 2 are shown. These are responses to pulses to −10 mV from a holding potential of −60 mV. Pulses were delivered at 0.5 Hz and had a duration of 200 msec. Pulse onset is indicated. The traces under CA are responses to pulse numbers 14, 10, 40, and 19 in (A) and 17, 21, 29, and 34 in (B). The traces under IO are responses to pulse numbers 1, 6, 8, and 16 in (A) and 6, 7, 25, and 62 in (B). Two channels are present in (B). (Row 2) A diary showing the proportion of open time per trace, *P* for *N* channels (*NP*) over the course of the experiment. (Row 3) Cumulative *NP*'s (cum *NP*) for the experiment. A 10-second gap occurred after excision. Cum NP_{IO}/cum NP_{CA} and survival as pulse number after excision are 0.14 and 20 for (A) and 2.14 and 63 for (B), respectively. Note the differences in the left-hand and right-hand ordinates in row 3.

now transiently increased after excision. The mean open times had average values of 1.4 msec before and 1.2 msec after excision into GTPγS, and the amplitudes did not change significantly from control ($n = 7$). Guanosine triphosphate (GTP) in equivalent concentrations was also effective (Table 1). The presence of ISO in the patch pipette was essential; without it, the effects were absent or greatly attenuated (Table 1). In the absence of receptor activation, GTPγS, while efficacious, is probably too slow as a G protein activator, and GTP is probably not sufficiently efficacious. Activation of G proteins by guanine nucleotides, especially those affected by β adrenoreceptors, is time, Mg^{2+}-, and temperature-dependent, and the process is accelerated by agonist occupancy of receptors (*10*). At the concentration of Mg^{2+} we used, lags in the activation of adenylyl cyclase by GTPγS are typically 15 to 20 minutes and are reduced by ISO to less than 10 seconds.

The stimulatory effect of GTP and the requirement that ISO be in the pipette pointed to G protein involvement. The most probable G protein was G_s, activation of which has as an obligatory sequel stimulation of adenylyl cyclase. Therefore we tested whether channel regulation by addition of GTPγS was dependent on local adenosine 3′,5′-monophosphate (cAMP) formation. A cAMP-dependent effect of G_s would require

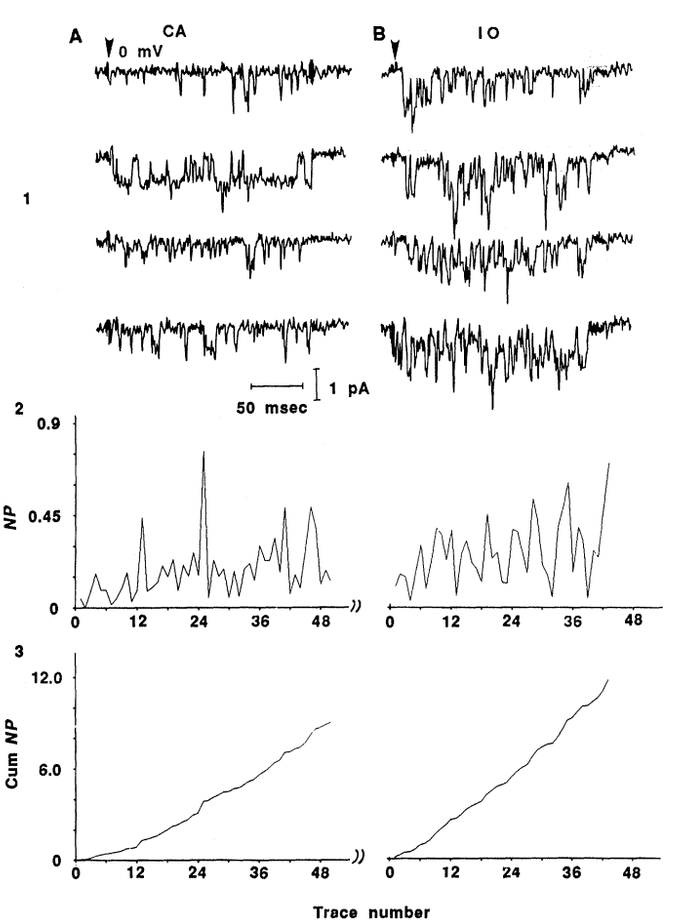

Fig. 2. Effects of the preactivated α subunit of G_s (α_s^*) on survival of Ca^{2+} channels in inside-out membrane patches excised from guinea pig ventricular myocytes. (Row 1) Representative single channel traces produced by steps to 0 mV from a holding potential of −50 mV at 0.5 Hz. The four responses under CA are to pulses 6, 16, 24, and 31, and under IO they are to pulses 4, 26, 29, and 42. The myocytes were incubated in $10^{-6}M$ ISO. G proteins were purified from human erythrocytes, activated with GTPγS, and their subunits separated as described (*2, 14, 15*). Additions were as in (*2*). (Row 2) Plots of *NP* and (Row 3) cumulative *NP* (cum *NP*). The effects of 50 p*M* α_s^* in the bath are under IO. Survival in the presence of α_s^* should be compared to the survival expected in its absence (IO in Fig. 1A and Table 1). Cum NP_{IO}/cum NP_{CA} and survival pulse number after excision are 1.12 and 42, respectively.

that the effects of GTPγS be (i) mimicked by supplying excess adenosine triphosphate (ATP) and cAMP, which bypass the adenylyl cyclase step and stimulate phosphorylation by the existing cAMP-activated protein kinase (PKA); (ii) blocked by a competitive inhibitor of adenylyl cyclase activity, adenosine 5′-*O*-(2-thio)diphosphate (ADPβS); (iii) blocked by protein kinase inhibitor (PKI) with concentrations that inhibited the increase in whole cell Ca^{2+} currents produced by ISO (*11*); and (iv) mimicked by local stimulation of adenylyl cyclase in a manner that does not involve activation of G_s (such as on addition of forskolin to the pipette). The results summarized in Table 1 show that we failed to obtain any of these effects. Involvement of protein kinase C (PKC) is improbable because this kinase requires much higher Ca^{2+} concentrations than the subnanomolar amounts we used. Nevertheless, in two experiments we tested the PKC activator tetradecanoyl phorbol acetate (TPA) ($10^{-6}M$) in the absence of added ATP and failed to observe any effect (*12*).

GTPγS also produced its stimulatory effects when the dihydropyridine agonist Bay K 8644 ($10^{-6}M$) replaced ISO in the bath (Table 1). Thus prior stimulation by cAMP-dependent phosphorylation was not essential.

We next tested the possibility that high threshold cardiac Ca^{2+} channels were regulated not only through phosphorylation by PKA but additionally by a direct, proximal effect of the G protein G_s. We examined the effects of G_s that had been purified from human erythrocytes and preactivated with GTPγS (*13*) (G_s^*), as well as the effect of its α subunit α_s^* after separation from βγ subunits with ion exchange chromatography (*13*). Both G_s^* and α_s^* reproduced the effects observed with the ISO-GTP combination, and neither effect required that ISO be in the patch pipette (Table 1 and Fig. 2). We used concentrations between 20 and 100 p*M* of either G_s^* or α_s^* with identical results. Unactivated G_s (three experiments) was ineffective as was preactivated G_k (six experiments), which directly stimulates mammalian atrial muscarinic K^+ channels (*14*). Because G_s^* is as effective as the much smaller guanine nucleotides in prolonging channel survival, the failure of PKA and PKI to affect channel survival (Table 1) could not have been due to physical diffusion barriers within the Ω-shaped membrane patches. Thus G protein regulation is specific, and, as for other G proteins, the specificity resides

Table 1. Effect of G protein activation and of activated G protein (G_s^* and α_s^*) on Ca^{2+} channel survival in excised membrane patches of adult guinea pig ventricle cells stimulated by phosphorylation (ISO) or Bay K 8644 (*20*). FSKL, forskolin; LPT, leupeptin.

Modulators added to		Relative Ca^{2+} channel activity before and after excision* (cum NP_{IO}/ cum NP_{CA})	Ca^{2+} channel survival† mean (number of pulses‡)	Number of patches analyzed
Pipette	Bath			
—	—	<<0.01	2 (1–5)	12
—	ISO		7 (3–16)	4
—	ISO, GTPγS	0.07 ± 0.06	9 (3–18)	6
ISO	ISO	0.15 ± 0.16	15 (5–29)	5
ISO	ISO, GTPγS	1.07 ± 0.60	78 (30–129)	7
ISO	ISO, GTP	0.98 ± 0.30	118 (48–200)	4
ISO	ISO, cAMP, ATP	0.17 ± 0.20	15 (8–25)	4
ISO	ISO, cAMP, ATP, GTPγS	1.4 ± 0.47	84 (50–132)	4
ISO	ISO, cAMP, ATP, PKA, LPT	0.26 ± 0.16	38 (20–60)	5
ISO	ISO, cAMP, ATP, PKA, LPT, GTPγS	1.52 ± 0.61	107 (61–182)	5
ISO, FSKL	ISO, ATP, FSKL	0.22 ± 0.11	21 (16–30)	4
ISO, FSKL	ISO, ATP, FSLK, GTPγS	1.35 ± 0.60	115 (34–165)	4
ISO	ISO, PKI, GTPγS	1.07 ± 0.63	84 (60–130)	4
ISO	ISO, ADPβS, GTPγS	0.93 ± 0.44	74 (60–83)	4
—	ISO, G_s^*	1.18 ± 0.33	74 (50–90)	4
—	ISO, α_s^*	1.08 ± 0.28	60 (41–106)	4
—	Bay K 8644	0.08 ± 0.06	10 (3–20)	8
—	Bay K 8644, GTPγS	—	15 (6–23)	5
ISO	Bay K 8644	0.09 ± 0.04	16 (5–33)	5
ISO	Bay K 8644, GTPγS	0.85 ± 0.18	77 (54–124)	8

*Mean ± SD. †Pulse rate at 0.5 Hz. ‡Numbers in parentheses indicate range.

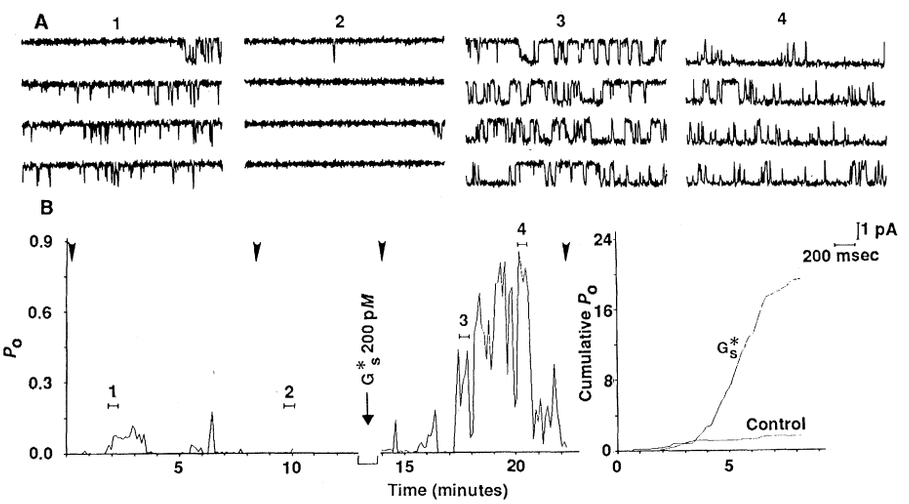

Fig. 3. Effects of G_s^* on single cardiac Ca^{2+} channels incorporated into a planar lipid bilayer (*23*). The number of functional Ca^{2+} channels incorporated into the bilayer was usually less than three and often one. As originally reported, cardiac sarcolemmal Ca^{2+} channels incorporated into this type of bilayer (*16*) had properties similar to those of high-threshold or L-type cardiac Ca^{2+} channels. In the presence of Bay K 8644 and with 100 m*M* Ba^{2+} as the charge carrier, the conductance between −50 and +20 mV was ~20 pS. The channel opened in bursts, and the probability of opening was voltage-dependent. Brief openings that dominate under control conditions could not be detected at the recording bandwidth, and the mean open times were fit to an exponential distribution ($\tau \sim 12 \pm 3$ msec, $n = 9$, at test potentials between 0 and +20 mV). The values are consistent with those obtained for Bay K 8644–stimulated channels in cell-attached patches (*24*). Current traces produced by depolarizing clamp steps to 0 mV from a holding potential of −40 mV are shown in (**A**) before (1 and 2) and after (3 and 4) addition of G_s^*. Pulses were applied every 30 seconds for 20 seconds. Leakage and capacitive currents were subtracted. Traces 1 to 4 were taken at the times indicated in (B) and are 2-second segments from the 20-second pulses. (**B**) The entire experimental record. Note the decrease in activity with time in control in (B) before addition of G_s^* (100 p*M*) to the trans chamber. The ordinate is given as P_o because N was 1 in this experiment. (**C**) Cumulative P_o's obtained between the arrows in (B). Cum P_o (G_s^*)/cum P_o (control) is 11.

in the α subunit. Although G_s seems the most likely candidate as a physiological regulator, other G proteins cannot be excluded (*15*); the lack of involvement of protein kinase C suggests that a G_p is not involved.

The cAMP-independent effect of the G protein on Ca^{2+} channel regulation may be due to the inhibition of a transition of the channel from a more activatable state, induced by phosphorylation (ISO in the bath) or by Bay K 8644 (phosphorylation-independent), to a less activatable state. This could be accomplished by an enzyme or other membrane protein responsible for the rapid loss of channel activity in the excised patch or by a direct interaction with the Ca^{2+} channel, causing it to be more resistant to an inactivating enzyme. An action of the G protein on a phosphatase (inhibitory) seems unlikely because the G protein is equally effective in prolonging the activity of ISO- and Bay K 8644–stimulated channels. Calcium-activated enzymes are also not likely to be involved because free Ca^{2+} was buffered to 10^{-8} *M* or less with EGTA.

To further examine the possibility of direct G protein–Ca^{2+} channel interaction, we studied the effects of G_s^* and α_s^* on Ca^{2+} channels from bovine cardiac sarcolemmal vesicles after their incorporation into lipid bilayers (*16*). Bay K 8644 was used to promote Ca^{2+} channel activity, which had the characteristic openings described in (*16*) (Fig. 3). After channel incorporation, activity produced by our pulsing protocol decreased slowly over periods of 5 to 10 minutes (*16*) (Fig. 3). Addition of G_s^* to the trans (intracellular) chamber produced a progressive increase in *NP* over the next 4 minutes followed by a reduction to levels well above those observed in control. The experiment ended when the bilayer broke down. The cumulative *NP*'s before and after G_s^* were also compared to emphasize the nearly logarithmic magnitude of the effect. Unitary current amplitudes were not changed but open times were prolonged from 8.2 to 29 msec and closed times were shortened from 98 to 55 msec. These values are distorted by the low-frequency response of the bilayer recording system and could result from either undetected closures or prolonged openings. G_s^* produced similar effects in three other experiments (the range of the ratios of cumulative *NP*'s was 5 to 8). At concentrations of 100 p*M*, α_s^* had the same effects as G_s^* ($n = 3$). Addition of G_s^* ($n = 4$) and α_s^* ($n = 4$) to the cis (extracellular) chamber had no detectable effects on Ca^{2+} channel activity. Addition to the trans chamber of the activated inhibitory G protein obtained from human erythrocytes (G_k^*), which stimulated atrial muscarinic K^+ channels, at concentrations between 100 and 400 p*M*, had no effects on Ca^{2+} channel activity ($n = 5$). Hence the stimulatory effects seem to be specific (at least between G_s^* and G_k^*), and the site of action is cytoplasmic.

The incorporated Ca^{2+} channels stimulated by G_s^* were either dephosphorylating slowly or did not require phosphorylation for channel function. G_s^* acted too quickly to be inhibiting dephosphorylation or a slowly progressive protease action. The complete absence of a high energy phosphate source precludes phosphorylation by the added G protein as a factor. Hence, by exclusion, the idea of a direct stimulatory effect of G_s^* on Ca^{2+} channels seems most plausible. In these experiments Ca^{2+} channels were kept in an activatable and hence G protein–susceptible state by Bay K 8644. Physiologically the channels can be made more activatable by phosphorylation with PKA (*11*). Phosphatases antagonize the ISO effect (*17*) and may be involved in channel inactivation (*18*). The existence of direct G protein regulation of Ca^{2+} channels, parallel and complementary to phosphorylation, could explain the lack of effect of PKI on basal Ca^{2+} channel activity in cardiac cells (*11*) and the cAMP-independent Ca^{2+} influx in adrenal glomerulosa cells on addition of adrenocorticotrophic hormone, which mediated stimulation of G_s^* (*19*). The relative importance of these dual pathways, channel phosphorylation and direct effects, have yet to be determined.

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bathing solution contained 110 m*M* potassium aspartate, 20 m*M* KCl, 2 m*M* EGTA, 2 m*M* $MgCl_2$, 20 m*M* glucose, 5 m*M* Hepes (*p*H 7.4 with tris), and the patch pipette contained 90 m*M* $BaCl_2$, 10 m*M* glucose, 10 m*M* Hepes (*p*H 7.4 with tris). L(−)-Isoprenaline (ISO) (Sigma) and GTPγS (tetralithium salt) (Boehringer Mannheim) were dissolved in distilled water as 10 m*M* stock solutions. All drugs and nucleotides were added either to the patch pipette solution or bath solution to obtain the final desired concentrations. External solutions were perfused through the chamber at 2 ml/min by gravity flow. Unitary currents were filtered with a 4-pole Bessel filter at 2 kHz, digitized at 5 kHz, and stored on a PDP 11/73 computer [H. D. Lux and A. M. Brown, *J. Gen. Physiol.* **83**, 727 (1984)]. Analyses of transitions were done on records filtered subsequently with a zero phase four-pole nonringing digital filter.

23. Bovine cardiac sarcolemmal vesicles were prepared and stored at −70°C [R. S. Slaughter, J. L. Sutko, J. P. Reeves, *J. Biol. Chem.* **258**, 3183 (1983); L. R. Jones, S. W. Maddock, H. R. Beach, *ibid.* **255**, 9771 (1980)]. Experiments were carried out at room temperature (20° to 22°C) in lipid bilayers formed from decane solutions of equimolar brain phosphatidylserine and phosphatidylethanolamine (Avanti Polar Lipid, Birmingham, AL). The cis chamber (500 μl) contained 50 m*M* NaCl, 100 m*M* $BaCl_2$, 2 m*M* $MgCl_2$, 10 m*M* Hepes (*p*H 7.4 with NaOH). The trans chamber (500 μl) contained the same solution as the cis chamber without $BaCl_2$. Bay K 8644 (1 μ*M*) was present on both sides. Vesicles were added to the cis chamber to a final concentration of 5 to 10 μg protein per milliliter. Incorporation occurred as for conventional right-side-out vesicles and depolarizing pulses opened channels more frequently. The cis chamber was connected to ground, and potentials were applied to the trans chamber. Thus, the trans chamber represents the intracellular side. The current conventions were those used in whole-cell recordings, and inward current (cis to trans) gave downward deflections. Current traces were recorded with a List EPC7 amplifier, filtered at 300 Hz (four-pole Bessel, low pass), and stored in a videocassette recorder. Data were digitized at sampling rates of 1 to 3 kHz and analyzed with a PDP 11/73 computer (*22*).
24. A. M. Brown, D. L. Kunze, A. Yatani, *Nature (London)* **311**, 570 (1984); P. Hess, J. B. Lansman, R. W. Tsien, *ibid.*, p. 538; S. Kokubun and H. Reuter, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4824 (1984).
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Technical Comments

Epilepsy Hypothesis

The report "Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy" by Robert S. Sloviter (*1*) demonstrates a loss of somatostatin-containing hilar neurons ipsilateral to perforant path stimulation. However, the report contains incomplete immunocytochemical results for γ-aminobutyric acid (GABA) neurons in the hilus of the dentate gyrus. The author does not appear to have replicated the findings of many investigators (*2–4*) who have shown large numbers of GABAergic hilar neurons. In fact, two of these studies (*3*) have shown that many somatostatin-containing neurons in the hilus are GABAergic. This finding was expected because many GABAergic hilar neurons resemble the morphology of somatostatin neurons in the hilus of the rat, and it is now clear that both GABAergic (*4*) and somatostatin-containing hilar neurons in the rat have commissural and associational projections. Therefore, the loss of somatostatin hilar neurons indicates that significant numbers of GABAergic hilar neurons are also degenerating.

It is possible that Sloviter's immunocytochemical results for GABAergic neurons in the hilus are related to the fixation protocol, in which a low concentration of glutaraldehyde (0.01%) was used. Although this fixative provides good staining for peptide-containing neurons, the antiserum to GABA is usually more effective with preparations that are fixed with higher concentrations of glutaraldehyde (*2, 3*). In order to use these same preparations to localize GABAergic neurons, it might be better to use an antiserum to glutamate decarboxylase (the synthesizing enzyme for GABA) that does not require glutaraldehyde in the fixative.

Sloviter interprets his results as indicating that GABAergic hilar neurons are not lost. Because he did not stain the normally large population of GABAergic neurons in the hilus, it is not known whether a significant change occurred in that population after stimulation of the perforant path. It is possible that such a change did occur, especially in light of the numerous degenerating hilar neurons on the stimulated side. Thus Sloviter's first conclusion, that the GABA-containing hilar neurons are impervious to the stimulation, could be incorrect. Since GABA and somatostatin are colocalized in many hilar neurons in the rat and cat (*3*), Sloviter's second and final conclusions also could be incorrect because the population of somatostatin-containing neurons that appears to be lost in this study would include many GABAergic neurons. Therefore, the proposed novel epilepsy hypothesis, which states that the loss of GABAergic neuron activation by hilar neurons on the stimulated side is the basis for the physiological loss of inhibition, is questionable.

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Response: Ribak asserts that I have not replicated the results of other investigators who have shown large numbers of hilar γ-aminobutyric acid (GABA)–containing neurons, and he cites an impressive number of studies to support his statement. In fact, the studies he cites do not support his assertion. Indeed, four of the citations say nothing whatever about the proportion of hilar neurons that are GABA- or glutamic acid decarboxylase (GAD)–positive and show few photomicrographs of the hilus (*1, 2*). Our results in the hippocampus with antiserum to GABA (*3, 4*) are identical to those of Ottersen and Storm-Mathisen (*5*), who used a different antiserum to GABA, and to those of Anderson and his colleagues (*2*), who used the same antiserum to GABA we used. Our results are also similar to those of Mugnaini and Oertel (*6*), who used antiserum to GAD. Our results differ significantly only from those of Seress and Ribak, who concluded that at least 60% of the cells of the dentate hilus are GABA neurons (*7*). Excluded from their analysis were the GABA- and GAD-positive basket cells within or subjacent to the granule cell layer. Immunocytochemical experiments conducted in this laboratory with antiserum to GABA, with the use of the high glutaraldehyde fixation Ribak suggests, show numerous hilar GABA neurons (*4*), but contradict Seress and Ribak's conclusion that a majority of hilar neurons are GABA neurons.

Ribak's second point is that other studies have shown that many hilar somatostatin-positive neurons are GABAergic and that therefore my finding that hilar somatostatin neurons have degenerated means that a loss of GABA neurons must have occurred. Only one study, by Schmechel and colleagues (*8*),