Organization	Address	Area of interest	Approximate funding	Type of funding
Union Pacific Foundation 212-826-8260	345 Park Avenue New York, NY 10154	Neurology and hearing	$10 000	Research grants
Van Ameringen Foundation 212-758-6221	680 Madison Avenue New York, NY 10021	Brain functions	$33 300	Research grants
Whitaker Foundation 717-763-1391	875 Poplar Church Road Camp Hill, PA 17011	Brain and spine	$141 000	Research grants
William Randolph Hearst Foundation 212-586-5404	888 Seventh Avenue New York, NY 10106	Hearing and speech	$20 000	Research grants
W. M. Keck Foundation 213-680-3833	555 South Flower Street Suite 4750 Los Angeles, CA 90071	Spinal cord injury, autoimmune diseases, deafness and varied neurosciences	$958 000	Research grants
Wood Kalb Foundation	One Rockefeller Plaza Suite 2100 New York, NY 10020	Alzheimer's disease/related disorders and neuropathology	$60 000	Research grants

reviews

G proteins as regulators of ion channel function

Kathleen Dunlap, George G. Holz and Stanley G. Rane

Virtually unknown just a decade ago, GTP-binding proteins (G proteins) have become a major focus of current research. This family of closely related proteins transduce extracellular signals (such as hormones, neurotransmitters and sensory stimuli) into effector responses[1,2]. It is now evident that ion channel permeability is one such effector response. In fact, the striking increase in the frequency of reports that demonstrate G protein-regulated ion channel function suggests that channels whose permeability mechanism can be altered by a G protein-mediated process may be more the rule than the exception. It is well-known that the cAMP-dependent modulation of ion channels is under the control of G proteins that regulate adenylate cyclase activity[3,4]. However recent studies demonstrate that G proteins also transduce agonist-induced changes in channel activity that do not involve adenylate cyclase. It is on this aspect of G protein signal transduction that this review will focus.

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Much of our information on the mechanisms underlying G protein function comes from research on G_s, the regulatory protein mediating hormonal stimulation of adenylate cyclase. According to one presently accepted model (for review see Ref. 5), G_s, in the non-activated state, exists as a heterotrimer of α-, β- and γ-subunits with GDP bound on the α-subunit (G–GDP). The interaction of agonist, receptor, and G–GDP accelerates the exchange of GTP for bound GDP. Binding of GTP by the α-subunit stimulates both the dissociation of G–GTP from the agonist–receptor complex, and the dissociation of the G protein itself into α-GTP (the active subunit with GTPase activity) and βγ (the regulatory dimer). Via a mechanism that remains to be identified, α-GTP promotes an increase in adenylate cyclase activity. The action of α-GTP is terminated through the α-subunit-mediated hydrolysis of GTP followed by reassociation of the α-subunit with free βγ to reform the inactive G–GDP. The agonist-induced activation of a G protein and its deactivation through hydrolysis of GTP is illustrated schematically by the cycle in Fig. 1.

This general scheme of events has been postulated to underlie the mechanism of action of other, more recently discovered G proteins (G_i and G_o)[6–8], although the receptors and, in some cases, the effector enzymes involved are different. In addition to its inhibitory role in the regulation of adenylate cyclase, a G_i-like protein may, for example, control the activity of other second messenger-generating enzymes such as phospholipase C or phospholipase A_2[9–12]. Similarly, it has been thought that G_o, a regulatory protein found in abundance in neural tissue (~1% of total protein), might also be linked to enzyme effectors other than adenylate cyclase but, until recently, the α-subunit of G_o has had no demonstrable physiological function. A recent paper by Hescheler *et al.*[13] indicates that α_o may play a pivotal role in regulating Ca^{2+} channel function in neurons. Furthermore, the recently reported co-localization of α_o immunoreactivity and phorbol ester binding in brain[14] suggests the possibility that physiological processes regulated by this G protein may be mediated by protein kinase C.

G proteins as regulators of Ca^{2+} channels

Ca^{2+} channels in neurons and neurosecretory cell lines are regulated by a variety of neurotransmitters and peptides[15]. Recent evidence suggests that, in some cases, these effects are mediated by G proteins. Pertussis toxin (PTX, a bacterial exotoxin that blocks the receptor-mediated activation of certain G proteins, notably G_i and G_o, through the ADP ribosylation of the α-subunit) interferes with the norepinephrine (NE) and GABA receptor-mediated inhibition of Ca^{2+} currents recorded from dorsal root ganglion (DRG) neurons[16]. Similarly, PTX also blocks

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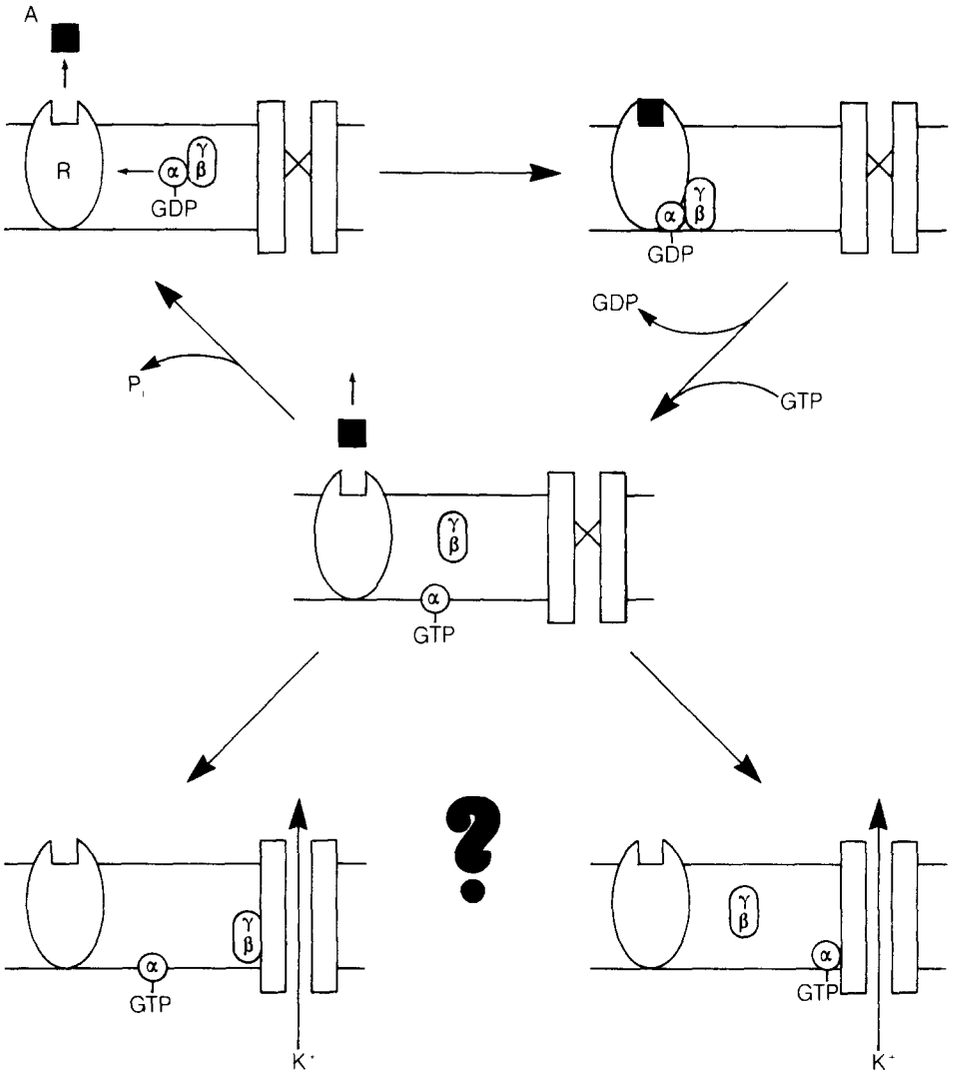

Fig. 1. *Schematic diagram of a cell membrane depicting the cycle of agonist-stimulated G protein activation and its deactivation through GTP hydrolysis. The lower part of the figure also illustrates two possible mechanisms for the G protein-mediated effects of acetylcholine on cardiac myocyte, inward rectifiying K^+ channels. A, agonist; R, receptor; α, α-subunit of G protein; βγ, βγ-dimer of G protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate.*

the inhibitory actions of the enkephalin analog D-Ala-D-Leu-enkephalin (DADLE) and somatostatin on Ca^{2+} currents recorded from the neuroblastoma–glioma cell line NG108-15, and the pituitary cell line AtT20, respectively[13,17]. As mentioned above, activation of G proteins is terminated by hydrolysis of the bound GTP. Intracellular application of the non-hydrolysable GTP analog, GTP-γ-S, converts the normally reversible responses to agonist in these cells into irreversible responses[13,17,18]. In contrast, intracellular application of the GDP analog GDP-β-S (which competes with GTP for the nucleotide binding site) blocks the inhibitory actions of NE and DADLE on Ca^{2+} currents recorded from DRG and NG108–15 cells, respectively[13,16]. Although these results argue that receptor-mediated inhibition of voltage-dependent Ca^{2+} channel function is indeed under the control of PTX-sensitive G proteins, the identities of the G proteins and the effector enzymes they might regulate remain to be determined.

Recent *in vivo* reconstitution experiments by Hescheler *et al.*[13] have sought to address this question. NG108-15 cells were treated with PTX at a concentration that reduced DADLE-induced inhibition of Ca^{2+} current to approximately 10% of control. Toxin-treated cells were then dialysed (under whole cell recording) with porcine brain G_o or G_i (as the heterotrimers or as isolated α-subunits, neither preactivated with GTP-γ-S) or transducin (G_t, a PTX-sensitive G protein purified from bovine retina). Dialysis with 0.4 nM α_o for 15 min produced a restoration of the enkephalin-induced inhibition of Ca^{2+} current to within 70% of control. On the basis of dose-response relationships, G_i was determined to be ten times less potent than α_o, and transducin was completely ineffective in reconstituting function in the PTX-treated cells.

These results provide preliminary evidence for a physiological role of G_o. However, this conclusion should be made with caution, since the authors have compared the potency of the G_i heterotrimer with that of the purified α-subunit from G_o. Furthermore, it is important to emphasize that, although the results of Heschler *et al.* establish that dialysis of NG108–15 cells with α_o can reconstitute function, they do not prove that G_o is the actual G protein (or α_o the active subunit) responsible for transduction of the enkephalin-induced inhibition of Ca^{2+} channel function in these cells. Future studies will undoubtedly use antibodies directed against identified G proteins to address this question more directly.

It will be of further interest to determine the identity of effector enzymes likely to be activated by G_o, as it is clear that adenylate cyclase, calmodulin, and guanylate cyclase do not play a role in the Ca^{2+} channel modulation reported for the above cells[13,16–20]. It is noteworthy in this regard that protein kinase C activators mimic the agonist-induced inhibition of Ca^{2+} currents in DRG neurons[21] and AtT20 cells (Lewis, D., pers. commun.). Therefore, it is possible that phospholipases and/or protein kinase C are targets for G protein regulation in neurons. In contrast to these transmitter effects on neuronal Ca^{2+} channels, acetylcholine-induced inhibition of Ca^{2+} channels in cardiac myocytes is thought to involve a decrease in cAMP levels, which results from G_i-mediated inhibition of adenylate cyclase[22] and/or activation of guanylate cyclase (and cGMP-stimulated phosphodiesterase)[23]. Thus, voltage-dependent Ca^{2+} channels appear to be targets for inhibition by multiple G proteins and second messengers. However, in addition to G protein regulation of Ca^{2+} channels via alterations in enzyme activity, the possibility of direct effects of G protein subunits on the channels must also be considered, particularly in light of recent findings for nerve and cardiac muscle K^+ channels discussed below.

G proteins as regulators of K^+ channels

Recent work on the muscarinic acetylcholine (ACh) receptor-activated K^+ permeability in atrial cardiac muscle suggests that intervening second messenger systems may not be necessary to some forms of G protein-mediated signal transduction. Results from several laboratories suggest that a G protein directly couples muscarinic receptor binding to alterations in K^+ channel function, producing an increase in the frequency of opening of an inwardly rectifying channel of 40–45 pS conductance[24]. (1) The response to ACh requires the presence of GTP[24–26]. (2) Non-hydrolysable analogs of GTP (GTP-γ-S and Gpp(NH)p) produce irreversible increases in K^+ channel activity[27] that are independent of agonist binding[24,25,28]. (3) PTX blocks the actions of ACh[24–26,28].

Although these experiments have established a primary role for a G protein in regulating cardiac K^+ channel function, attempts at identifying likely second messenger enzyme systems regulated by the G protein have yielded negative results. That is, second messengers such as cAMP, cGMP, diacylglycerol, and Ca^{2+} do not appear to mediate these actions of acetylcholine[28,29]. This has led investigators to postulate a direct action of the G protein on the K^+ channel without the involvement of an effector enzyme. As a test of this hypothesis, three laboratories have used the single channel recording technique to study the effects of nucleotides and/or purified G proteins on K^+ channel activity in inside-out patches of atrial membrane[24,25,28]. In this configuration, the cytoplasmic face of the membrane can be bathed in an experimental solution, in the absence of the normal metabolic machinery of the cell's cytoplasm. These studies have shown that stimulation of K^+ channel activity by ACh can be mimicked by direct application (to the cytoplasmic side of the membrane) of either non-hydrolysable analogs of GTP or the active components of the G proteins themselves (see below). This indicates that cytoplasmic second messengers are not necessary for the action of ACh. Although this is consistent with the hypothesized direct effect of the G protein on K^+ channels, a novel membrane-associated second messenger system cannot, as yet, be ruled out.

The identity of the G protein that mediates the effect of ACh on K^+ channel function has been addressed by Yatani *et al.*[28] and by Logothetis *et al.*[25] The former authors suggest that a 'G_i-type' protein (containing a 40 kDa α-subunit) plays a primary role in the transduction process. Purified from human erythrocytes as the heterotrimer and activated by GTP-γ-S, this protein (which they call G_k) produced a sustained stimulation of K^+ channel activity in inside-out membrane patches (even those pre-treated with PTX) when applied to the cytoplasmic face. Lower concentrations of the G protein (0.5–2 pM) promoted the appearance of predominantly low conductance (22 pS) channels, while higher concentrations resulted in the activation of the 40 pS channel described originally by Kurachi *et al.* Although similar effects were observed with application of purified, GTP-γ-S-activated bovine brain G_o, the effect of G_k was 20–100 times more potent on a per mole basis, while activated G_s was completely ineffective at 2 nM. The authors suggest that, since these three G proteins are known to differ in their α-subunits (while the βγ dimer is shared by all), the data support the notion that α_k mediates the action of ACh.

Logothetis *et al.* have performed similar experiments using G_i and G_o. Surprisingly, these authors suggest that the α-subunit is not the active moiety. They report that purified α-subunits from bovine brain G_i (41 kDa) and G_o (39 kDa) were ineffective in stimulating the 40 pS K^+ channel activity in inside-out patches of atrial membrane. However, introduction of the βγ-dimer (also purified from bovine brain) resulted in a dramatic increase in the frequency of channel openings, an effect that could be reversed by pre-treating, and presumably buffering, the βγ preparation with purified α-subunit. Thus, the authors conclude that, in contrast to the accepted mechanism of action of G_s, the moiety responsible for transducing the action of ACh on the 40 pS K^+ channel appears to be βγ, while the α-subunit plays a regulatory role. This idea is not incompatible with the results of Yatani *et al.*, since the latter authors did not test the effects

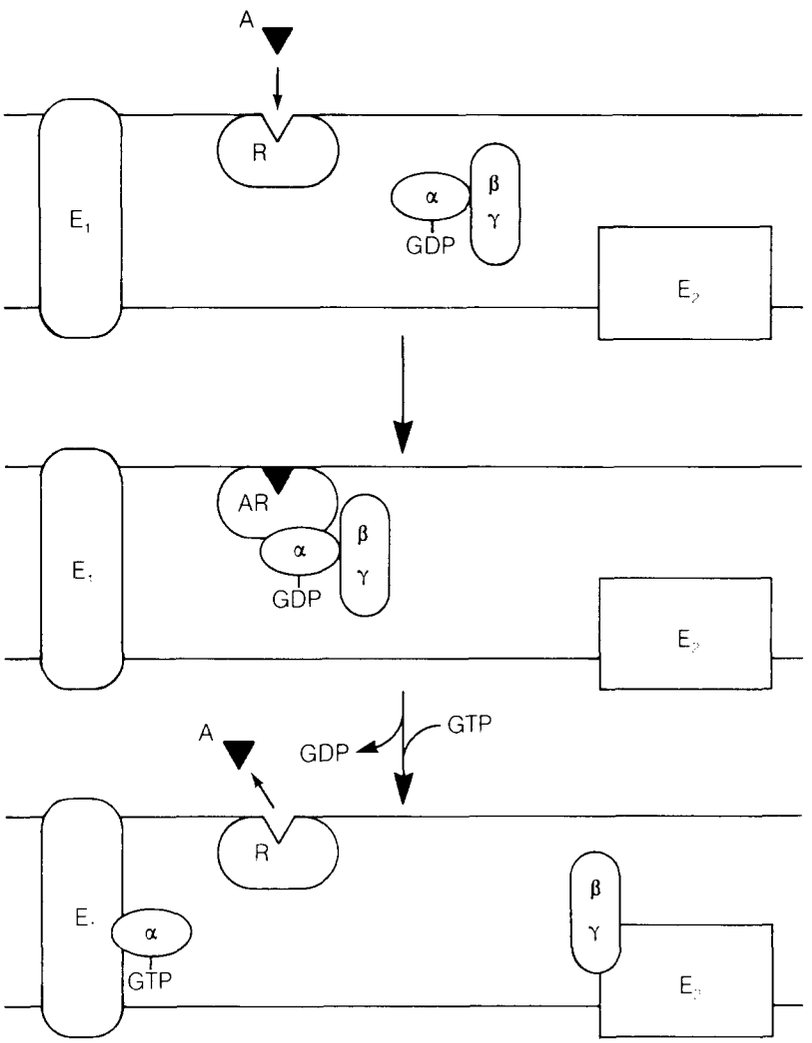

Fig. 2. *A hypothetical scheme that depicts a single G protein activating two different transduction pathways. Two effector molecules, E1 and E2, are differentially activated by the separate subunits (α and βγ) following dissociation of the G protein. Among a variety of potential target molecules, these effectors could be membrane channels or second messenger-generating enzymes (e.g. phospholipase C or adenylate cyclase). Other abbreviations are the same as in Fig. 1.*

of the subunits separately. Intriguing as it is, this suggestion that βγ might play the active role in mediating regulation of K^+ channels by G proteins must be confirmed by further studies; experiments to date have not ruled out the alternative possibility that application of βγ reverses a persistent K^+ channel inhibition induced by endogenous free α-subunit. Additionally, in light of the fact that picomolar concentrations of G_k can promote increased openings of the 40 pS K^+ channel[28], the purity of the brain βγ preparation will be a critical consideration for future studies.

A number of issues remain to be addressed. If βγ is, in fact, the active subunit, then it must be concluded that βγ from erythrocyte G_k is more effective than the (presumably) heterogeneous βγ isolated from brain, and that βγ of G_s is functionally different from βγ of G_k (since Yatani *et al.* report that activated G_s does not stimulate K^+ channel activity). On the other hand, if α is the active subunit, then it must be concluded that α_k (defined as α_i purified from erythrocytes) is different from α_i purified from brain (since Logothetis *et al.* reported no effects of α_i on K^+ channel function). Further studies utilizing isolated subunits from extensively purified G protein preparations will be necessary to begin to sort out answers to these important problems.

A direct action of βγ is not without precedent in the literature. Katada *et al.* and Enomoto and Asakawa have recently suggested that βγ from rat brain G_i or G_o exhibits a direct inhibitory effect on partially purified adenylate cyclase activity from rat brain[30,31]. It remains to be determined if this represents a general mechanism underlying the actions of G proteins in many systems. These observations have particular significance for studies on neuronal agonist-regulated K^+ channels. Alterations in K^+ channel function have, in some instances, been postulated to result from a direct action of the G protein on the channel itself[32,33], whereas in others, channel activity may be controlled by a G protein-regulated effector enzyme[34,35].

Given the ubiquitous nature of G proteins as signal transducers for receptor-linked effector responses, it is perhaps not surprising that receptor-induced alterations in voltage-dependent channel function are also mediated by G proteins. What may be more surprising is the wealth of information that has, in the course of the last 18 months, accrued from work in this area. Not only has it led to identifying a possible function of G_o in neurons, it has also suggested that both α- and βγ-subunits of G proteins have regulatory as well as direct roles in signal transduction. One interesting possibility that emerges from this speculation is that, upon dissociation, the α-subunit and βγ-subunit may mediate separate transduction pathways (as diagrammed in Fig. 2). At the very least, results from these studies on voltage-sensitive channel modulation suggest that G protein function is far more complicated than previously imagined.

Fact Sheet on Batten's Disease

Batten's disease is an inherited disorder of the nervous system, appearing in infancy and childhood and leading to dementia and loss of motor abilities. These changes are associated with a build-up of fatty pigments in cells of the brain, eye and other tissues. Current research is aimed at understanding the causes of this abnormal storage and the nature of the genetic deficit.

Single copies of the fact sheet are available free of charge from the Office of Scientific and Health Reports, NINCDS, Building 31, Room 8A16, National Institutes of Health, Bethesda, MD 20892, USA.

Selected references

1 Gilman, A. G. (1986) *Trends Neurosci.* 9, 460–463
2 Stryer, L. and Bourne, H. R. (1986) *Annu. Rev. Cell Biol.* 2, 391–419
3 Shain, W. and Carpenter, D. O. (1981) *Int. Rev. Neurobiol.* 22, 205–250
4 Lemos, J. R. and Levitan, I. B. (1984) *J. Gen. Physiol.* 83, 269–285
5 Birnbaumer, L., Codina, J., Mattera, R., Cerione, R. A., Hildebrandt, J. D., Sunyer, T., Rojas, F. J., Caron, M. G., Lefkowitz, R. J. and Iyengar, R. (1985) in *Molecular Mechanisms of Transmembrane Signalling* (Cohen, and Houslay, M., eds), pp. 131–182, Elsevier
6 Bokoch, G. M., Katada, T., Northup, J. K., Ui, M. and Gilman, A. F. (1984) *J. Biol. Chem.* 259, 3560–3567
7 Neer, E. J., Lok, J. M. and Wolf, L. G. (1984) *J. Biol. Chem.* 259, 14222–14229
8 Sternweis, P. C. and Robishaw, J. D. (1984) *J. Biol. Chem.* 259, 13806–13813
9 Burch, R. M., Luini, A. and Axelrod, J. (1986) *Proc. Natl Acad. Sci. USA* 83, 7201–7205
10 Kiruchì, A., Kozawa, O., Kaibuchi, K., Katada, T., Ui, M. and Takai, Y. (1986) *J. Biol. Chem.* 261, 11558–11562
11 Nakamura, T. and Ui, M. (1985) *J. Biol. Chem.* 260, 3584–3593
12 Ohta, H., Okajima, F. and Ui, M. (1985) *J. Biol. Chem.* 260, 15771–15780
13 Hescheler, J., Rosenthal, W., Trautwein, W. and Schultz, G. (1987) *Nature* 325, 445–447
14 Worley, P. F., Baraban, J. M., Van Dop, C., Neer, E. J. and Snyder, S. H. (1986) *Proc. Natl Acad. Sci. USA* 83, 4561–4565
15 Reuter, H. (1983) *Nature* 301, 569–574
16 Holz, G. G., Rane, S. G. and Dunlap, K. (1986) *Nature* 319, 670–672
17 Lewis, D. L., Weight, F. F. and Luini, A. (1986) *Proc. Natl Acad. Sci. USA* 83, 9035–9039
18 Scott, R. H. and Dolphin, A. C. (1986) *Neurosci. Lett.* 69, 59–64
19 Forscher, P. and Oxford, G. S. (1985) *J. Gen. Physiol.* 85, 743–763
20 Forscher, P., Oxford, G. S. and Schulz, D. (1986) *J. Physiol. (London)* 379, 131–144
21 Rane, S. G. and Dunlap, K. (1986) *Proc. Natl Acad. Sci. USA* 83, 184–188
22 Hescheler, J., Kameyama, M. and Trautwein, W. (1986) *Pflug. Arch.* 407, 182–189
23 Hartzell, H. C. and Fischmeister, R. (1986) *Nature* 323, 273–275
24 Kurachi, Y., Nakajima, T. and Sugimoto, T. (1986) *Pflug. Arch.* 407, 264–274
25 Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J. and Clapham, D. E. (1987) *Nature* 325, 321–326
26 Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M. and Hille, B. (1985) *Nature* 317, 536–538
27 Breitwieser, G. E. and Szabo, G. (1985) *Nature* 317, 538–540
28 Yatani, A., Codina, J., Brown, A. M. and Birnbaumer, L. (1987) *Science* 235, 207–211
29 Trautwein, W., Taniguchi, J. and Noma, A. (1982) *Pflug. Arch.* 392, 307–314
30 Katada, T., Oinuma, M. and Ui, M. (1986) *J. Biol. Chem.* 261, 5215–5221
31 Enomoto, K. and Asakawa, T. (1986) *FEBS Lett.* 202, 63–68
32 Andrade, R., Malenka, R. C. and Nicoll, R. A. (1986) *Science* 234, 1261–1265
33 Sasaki, K. and Sato, M. (1987) *Nature* 325, 259–262
34 Aghajanian, G. K. and Wang, Y. Y. (1986) *Brain Res.* 371, 390–394
35 Higashida, H., Streaty, R. A., Klee, W. and Nirenberg, M. (1986) *Proc. Natl Acad Sci. USA* 83, 942–946