Roles of G Proteins in Coupling of Receptors to Ionic Channels and Other Effector Systems

Lutz Birnbaumer, Joel Abramowitz, Atsuko Yatani, Koji Okabe, Rafael Mattera, Rolf Graf, Jack Sanford, Juan Codina, and Arthur M. Brown

ABSTRACT

Guanine nucleotide binding (G) proteins are heterotrimers that couple a wide range of receptors to ionic channels. The coupling may be indirect, via cytoplasmic agents, or direct, as has been shown for two K^+ channels and two Ca^{2+} channels. One example of direct G protein gating is the atrial muscarinic K^+ channel K^+[ACh], an inwardly rectifying K^+ channel with a slope conductance of 40 pS in symmetrical isotonic K^+ solutions and a mean open lifetime of 1.4 ms at potentials between -40 and -100 mV. Another is the clonal GH_3 muscarinic or somatostatin K^+ channel, also inwardly rectifying but with a slope conductance of 55 pS. A G protein, G_k, purified from human red blood cells (hRBC) activates K^+[ACh] channels at subpicomolar concentrations; its α subunit is equipotent. Except for being irreversible, their effects on gating precisely mimic physiological gating produced by muscarinic agonists. The α_k effects are general and are similar in atria from adult guinea pig, neonatal rat, and chick embryo. The hydrophilic $\beta\gamma$ from transducin has no effect while hydrophobic $\beta\gamma$ from brain, hRBCs, or retina has effects at nanomolar concentrations which in our hands cannot be dissociated from detergent effects. An anti-α_k monoclonal antibody blocks muscarinic activation, supporting the concept that the physiological mediator is the α subunit not the $\beta\gamma$ dimer. The techniques of molecular biology are now being used to specify G protein gating. A "bacterial" $\alpha_{i\text{-}3}$ expressed in *Escherichia coli* using a pT7 expression system mimics the gating produced by hRBC α_k.

I. INTRODUCTION

G proteins play a central role in coupling receptors to effector systems (Figure 1). By the latest count about 85 distinct receptors, identified by pharmacological and/or molecular biological means, are coupled to effector functions by G proteins (e.g., Table 1). The number of G proteins carrying out this task is rather small in comparison, comprising 12 known and possibly 4 to 5 as yet to be discovered distinct Gα (Table 2). However, the recognition of their complexity is increasing with the discovery of up to 4 distinct β and at least 3 γ subunits that associate with various αs (Table 3). Like receptors, which are increasing in number rapidly, effectors affected by the activated forms of G proteins are also increasing (Table 4),

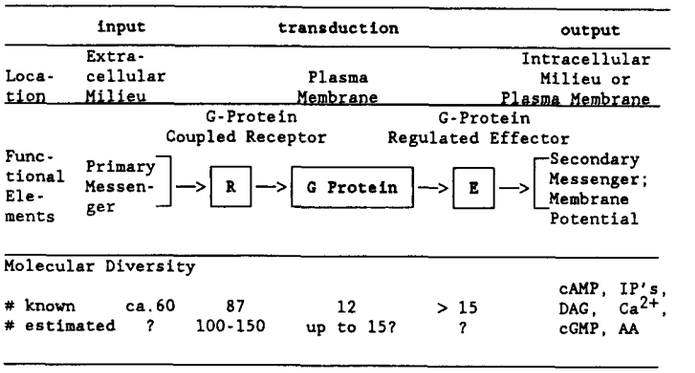


FIGURE 1. Flow of information through G protein-dependent signal transduction systems as found in vertebrates.

most notably through the discovery in 1986—1987 that ionic channels form part of the family of molecules regulated by G proteins. Using cell-free systems, such as provided by excision of membrane patches from cells and incorporation of plasma membrane vesicles into lipid bilayers, it was shown that ionic channels are indeed regulated by activated G proteins. Some of these channels had long before been predicted to be under the control of G protein-coupled receptors by means other than soluble second messengers. The mechanism by which G proteins regulate some of these channels is at the very least "mem-

L. Birnbaumer earned his Ph.D. at the University of Buenos Aires, Buenos Aires, Argentina. Presently, Dr. Birnbaumer is Professor, Departments of Cell Biology and Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas. **J. Abramowitz** earned his Ph.D. at Wayne State University, Detroit, Michigan. Dr. Abramowitz is an Associate, Departments of Medicine and Cell Biology, Baylor College of Medicine, Houston, Texas. **A. Yatani** earned her Ph.D. at the University of Tokyo, Japan. She is currently a Research Associate Professor of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas. **K. Okabe** earned an M.D. at the University of Tokyo, Japan. Dr. Okabe is a Research Associate at the Baylor College of Medicine, Houston, Texas. **R. Mattera** earned his Ph.D. at the University of Buenos Aires and is currently an Assistant Professor of Physiology at Case Western Reserve University School of Medicine. **R. Graf** has a Ph.D. from the University of Zürich, Switzerland. Dr. Graf is a Research Associate at the Baylor College of Medicine, Houston, Texas. **J. Sanford** has a Ph.D. from Tufts University, Boston, Massachusetts. Dr. Sanford is a Research Associate at the Baylor College of Medicine, Houston, Texas. **J. Codina** earned his Ph.D. at the University Complutense, Alcalá de Henares, Spain. Dr. Codina is a Research Assistant Professor of Cell Biology at the Baylor College of Medicine, Houston, Texas. **A. M. Brown** earned an M.D. at the University of Manitoba, Winnipeg, Canada and a Ph.D. at the University of London, England. Dr. Brown is Professor and Chairman, Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas.

Table 1
Examples of Receptors Acting on Cells via G proteins

Type of receptor	Membrane function/system affected	Effect	Coupling protein involved	Examples of target cells(s)/organs
		Neurotransmitters		
Adrenergic				
beta-1	AC	Stimulation	G_s	Heart (68), fat (62)
	Ca channel	Stimulation	G_s	Heart (4), skeletal muscle (5)
beta-2	AC	Stimulation	G_s	Liver (69), lung (70), lymphoid cells (71)
alpha-1	PhL C	Stimulation	G_{plc}	Smooth muscle (72), liver (73, 74)
	PhL A_2	Stimulation	G_{pla}	FRTL-5 cells (75)
	PhL D	Stimulation	G_{pld}	Liver (76)
alpha-2A, -2B	AC	Inhibition	G_i	Platelet (77, 78), fat (79, 80) liver (81)
	Ca channel	Closing	G_o (G_p?)	Symp. presynapse (24, 82)
Dopamine				
D-1	AC	Stimulation	G_s	Caudate Nucleus (83), parathyroid (84)
D-2	AC	Inhibition	G_i	Pituitary lactotrophs (85) and melanotrophs (86)
Acetylcholine				
Muscarinic M_1-type (M_1, M_3, M_5)	PhL C	Stimulation	G_{plc}	Pancreatic acinar cell (87), parotid (88), CNS (88)
	K channel (M)	Closing	?	CNS, Symp. ganglia (89)
Muscarinic M_2-type (M_2, M_4)	AC	Inhibition	G_s	Heart (68, 90)
	K channel	Opening	G_k (G_i)	Heart (2), CNS (91), lactotroph (3)
$GABA_B$	Ca channel	Closing	G_o (G_p?)	Dorsal root ganglia (83, 25)
	K channel	Opening	G_k (G_o?)	Pyramidal cells (92)
	AC	Inhibition	G_i	CNS (93)
Purinergic P1				
Adenosine A-1 or Ri	AC	Inhibition	G_i	CNS (94), fat (95)
	K channel	Opening	G_k (G_i)	heart (96)
Adenosine A-2 or Ra	AC	Stimulation	G_s	Fat (97), kidney (98), CNS (99)
Purinergic P_{2X} and P_{2Y}	PhL C (PIP_2)	Stimulation	G_{plc}	Turkey erythrocytes (100)
	PhL C (PC)	Stimulation	G-(?)	Liver (101)
	PhL D	Stimulation	G-(?)	Liver (76)
Serotonin (5HT)				
S-1a (5HT-1a)	AC	Inhibition	G_i	Pyramidal cells, CNS (102)
	K channel	Opening	G_k(G_i?,G_o?)	Pyramidal cells (103)
S-1c (5HT-1c)	PhL C	Stimulation	G_{plc}	Blowfly salivary gland (88), smooth muscle (103)
S-2 (5HT-2)	AC	Stimulation	G_s	Skeletal muscle (104), CNS (105)
	Ca channel	Closing	G_o (G_p?)	Dorsal root ganglia (82)
Histamine				
H-1	PhL C	Stimulation	G_{plc}	CNS (106), chromaffin cells (107)
H-2	AC	Stimulation	G_s	Heart (108), CNS (109), gastric mucosa (110)
		Peptide Hormones		
Pituitary				
Adrenocorticotropin (ACTH)	AC	Stimulation	G_s	Adrenal cortex (111)
	Ca channel	Opening	G_s(?)	Adrenal glomerulosa (112)
	PhL A_2	Stimulation	G-(?)	Adrenal cortex (113)
Opioid (mu, kappa, delta)	AC	inhibition	G_i	NG-108 (114, 115), luteal (116)
	Ca Channel	Closing	G_o (G_p?)	NG-108 (29), dorsal root ganglia (117, 118)
	K channel	Closing	G_k (G_i?)	CNS (119)
Luteinizing hormone (LH)	AC	Stimulation	G_s	Granulosa (120), luteal (121), Leydig (122)
	PhL C	Stimulation	G_p	Ovary (123)
Follicle stimulating hormone (FSH)	AC	Stimulation	G_s	Granulosa (120), Sertoli (122)
Thyrotropin (TSH)	AC	Stimulation	G_s	Thyroid (124)
Melanocyte stimulating hormone (MSH)	AC	Stimulation	G_s	Melanocytes (125)
Hypothalamic				
Corticotropin releasing hormone (CRF)	AC	Stimulation	G_s	Corticotroph (126)

Table 1 (continued)
Examples of Receptors Acting on Cells via G proteins

Type of receptor	Membrane function/system affected	Effect	Coupling protein involved	Examples of target cells(s)/organs
Growth hormone releasing hormone (GRF)	AC	Stimulation	G_s	Somatotroph (127, 128)
Gonadotropin releasing hormone (GnRH)	PhL A_2	Stimulation	G_{pla}	Gonadotroph (129), granulosa (130), Leydig (131)
	PhL C	Stimulation	G_{plc}	Gonadotroph (132), granulosa (33), luteal (134), Leydig (131)
	Ca channel	Opening	G_i-type	GH_3 (31)
Thyrotropin releasing hormone (TRH)	PhLC	Stimulation	G_{plc}	Lactotroph (135, 136), thyrotroph (137)
	AC	Inhibition	G_i	GH_4C_1 (138)
Somatostatin (SST or SRIF)	AC	Inhibition	G_i	Lactotrophs (139), somatotrophs (140), corticotrophs (141) lymphoid cells (142), liver (143), heart (143), renal cortex (143)
	K channel	Opening	G (G_i?)	Lactotrophs (3)
	Ca channel	Closing	?	Corticotrophs (28)
Vasopressin	PhL C	Stimulation	G_{plc}	Smooth muscle, liver (73), sympath. ganglia (144)
V-1a (vasopressor, glycogenolytic)				
	PhL D	Stimulation	G-(?)	Liver (76)
	PhL A_2	Stimulation	G-(?)	Renal mesangial cells (145)
	AC	Inhibition	G_i	liver (146)
V-1b (pituitary)	PhL C	Stimulation	G_{plc}	Pituitary (147)
V-2 (antidiuretic)	AC	Stimulation	G_s	Distal and collecting tubule (148)
Oxytocin	PhL C	Stimulation	G_{plc}	Uterus (149)
Other hormones				
Chorionic gonadotropin	AC	Stimulation	G_s	luteal (150), Leydig (151)
Glucagon	AC	Stimulation	G_s	Liver (152), fat (62), heart (153, 154), pancreatic *beta*-cells (155)
	Ca pump	Inhibition	G_s(?)	Liver (156)
	PhL C	Stimulation	?	Liver (157)
Cholecystokinin (CCK)	AC	Stimulation	G_s	Pancreatic acini (158)
	PhL C	Stimulation	G_{plc}	Pancreatic acini (159)
Secretin	AC	Stimulation	G_s	Fat (62) pancreatic acini (160)
Vasoactive intestinal peptide (VIP)	AC	Stimulation	G_s	CNS (161), pancreatic acini (160), intestinal mucosa (162)
	PhL C	Stimulation	G_{plc}	Sensory ganglia (163)
Parathyroid hormone (PTH)	AC	Stimulation	G_s	Renal cortex (164)
	PhL C	Stimulation	G_{plc}	Renal cortex (165)
Angiotensin II	PhL C	Stimulation	G_{plc}	Glomerulosa cells (166), smooth muscle (167)
	AC	Inhibition	G_i	Liver (163), glomerulosa cells (168), Leydig cells (169), renal cortex (170)
	Ca channel	Stimulation	G_i-type	Y1 adrenal cells (30)
	PhL A_2	Stimulation	G-(?)	Renal medulla (171), fibroblasts (172)
	PhL D	Stimulation	G-(?)	Liver (76)
Calcitonin	AC	Stimuation	G_s	Renal cortex (173)
Calcitonin gene-related peptide	AC	Stimulation	G_s	Skeletal muscle (174)
	PhL C	Stimulation	G_{plc}	Skeletal muscle (175)
Other Regulatory Factors				
Chemoattractant (fMet-Leu-Phe or fMLP)	PhL C	Stimulation	G_{plc}	Neutrophils (176)
	PhL A_2	Stimulation	G-(?)	Neutrophils (177)
Thrombin	PhL C	Stimulation	G_{plc}	Platelets (178), fibroblasts (172)
	AC	Inhibition	G_i	Fibroblasts (172)
	PhL A_2	Stimulation	G-(?)	Platelets (179)

Table 1 (continued)
Examples of Receptors Acting on Cells via G proteins

Type of receptor	Membrane function/system affected	Effect	Coupling protein involved	Examples of target cells(s)/organs
Bombesin	PhL C	Stimulation	G_{plc}	Fibroblasts (180)
Gastrin releasing peptide	PhL C	Stimulation	G_{plc}	Fibroblasts (180)
Platelet-derived growth factor	PhL C	Stimulation	G_{plc}	Fibroblasts (181)
IgE	PhL C	Stimulation	G_{plc}	Mast cells (182)
	PhL A_2	Stimulation	G-(?)	Mast cells (183)
Bradykinin	PhL C	Stimulation	G_{plc}	Fibroblasts (180), NG-108 (184), endothelial cells (185) renal papilla (186)
	PhL A_2	Stimulation	G_{pla}	Fibroblasts (172), endothel. cells (187), kidney (188)
	K channel	Stimulation	G_k (G_i?)	NG-108 (189)
	AC	Inhibition	G_i	NG-108 (184), fibroblasts (172)
Neurokinin/tachykinin NK1 (substance P)	PhL C	Stimulation	G_{plc}	CNS (190), smooth muscle (190) salivary gland (191)
	PhL A_2	Stimulation	G-(?)	Smooth muscle (192)
Neuropeptide Y	AC	Inhibition	G_i	Heart (193)
	K channels	Stimulation	G_k	Heart (261)
	Ca channels	Inhibition	G_o	Sensory ganglia (26)
Peptide YY	?	?	?	CNS (194)
Tumor necrosis factor (TNF)	?	?	?	Monocytes (195)
Colony stimulating factor (CSF-1)	?	?	?	Monocytes (196)
Interleukin-1	PhL C (PC)	Stimulation	G(?)	T-cells (197)
Neurotensin	PhL C	Stimulation	G_{plc}	CNS (198)
	AC	Inhibition	G_i	Neuroblastoma (199)
Atrial naturietic factor	AC	Inhibition	G_i	Aorta (200)
Epidermal growth factor	PhL D	Stimulation	G-(?)	Liver (76)
Phosphatidic acid	PhL C	Stimulation	G_{plc}	A431 carcinoma cells (201)
Platelet activating factor (PAF)	PhL C	Stimulation	G_{plc}	Platelets (202), liver (203)
	PhL A_2	Stimulation	G-(?)	Fibroblasts (172)
	AC	Inhibition	G_i	Fibroblasts (172)
Galanin	AC	Inhibition	G_i	Pancreatic *beta*-cells (204)
Kyotorphin	PhL C	Stimulation	G_{plc}, G_o?	CNS (205)
		Prostanoids		
Prostaglandin E_1, E_2	AC	Inhibition	G_i	Fat (206), kidney (207)
Prostaglandin $F_{2\,alpha}$	PhL C	Stimulation	G_{plc}	Luteal cells (208)
Prostacyclin (PGI_2) PGE_1, PGE_2	AC	Stimulation	G_s	Luteal cells (209, 210), kidney (211)
Leukotriene D_4, C_4	PhL A_2	Stimulation	G_{pla}	Endothelial cells (212, 213)
	PhL C	Stimulation	G_{plc}	RBL-1 cells (214)
		Sensory		
Light (Rhodopsins)	cGMP-PDE	Stimulation	$Tr(G_{t\text{-}r})$	Retinal rod cells (night) (215)
	cGMP-PDE	Stimulation	$Tc(G_{t\text{-}c})$	Retinal cone cells (color) (216a)
Olfactory signals	AC	Stimulation	G_{olf}	Olfactory cilia (216a)
	PhL C	Stimulation	G_p?	Olfactory cilia (217)

Note: AC: adenylyl cyclase: PhL C: unless denoted otherwise, phospholipase C with specificity for phosphatidylinositol bisphosphate; PhL A_2: phospholipase A_2 (substrate specificity unknown); PIP_2: phosphatidylinositol bisphosphate; PC: phosphatidylcholine.

brane delimited'' and independent of any phosphorylation event or of changes in cytoplasmic levels of second messengers such as cAMP, Ca^{2+}, or IP_3 and is very likely due to direct interaction of the G protein α subunit and the channel proper (for review of initial findings, see Reference 1). Our group was prominent in providing some of the initial as well as subsequent supporting data for these conclusions.[2-12] The issue whether $\beta\gamma$ dimers stimulate K^+ channel activity in inside-out mem-

Table 2
Diversity of Mammalian G Protein α Subunits

Name	Purified[a]	Cloned[a]	Function(s)[a] Identified
α_s (1 gene, 4 splice variants)	Yes	Yes	Yes: 3[b]
3 α_is (3 genes: α_i1, α_i2, α_i3)[c]	Yes	Yes	Yes
α_{o1} (1 gene: α_o1)	Yes	Yes	Yes (?)[d]
α_{o2} (splice variant of α_o gene ?)	Yes	No	No
α_t-rod	Yes	Yes	Yes
α_t-cone	No	Yes	Inferred
α_{olf}	No	Yes	Inferred
$\alpha_{z/x}$	Yes	Yes	No
"α_p"-PhLC[e]	?, No[f]	?, No[f]	Yes
"α_p"-PhLA$_2$[e]	?, No[f]	?, No[f]	Yes

[a] For detailed referencing see Birnbaumer et al.[218]
[b] See Table 3.
[c] Named in chronologic order of cloning (Suki et al.[219]).
[d] Stimulatory roles in both K^+ channel (Yatani et al.[7]) and phospholipase C regulation (Kikuchi et al.[220]) have been reported.
[e] PTX sensitive and PTX insensitive activities have been reported, heterotrimeric nature of the G protein involved is inferred from PTX snesitivity and from inhibition of "G_p"-mediated activities by βγ dimers (Moriarty et al.[221]). For detailed discussion see Birnbaumer et al.[222]
[f] The PTX sensitive form may have been purified/cloned, but the PTX-insensitive forms are unknown.

Table 3
Diversity in Subunit Composition of G Protein βγ Dimers[a]

		Refs.
β Subunits: β_1 (β_{36}), β_2 (β_{35}), β_3 (migration unknown)		
4 cloned		
	β1	223-225
	β2	226-228
	β3	229
	β4	259
	2 seen on SDS-PAGE	227,230,231,260
γ Subunits		
γ_T-1	(10 kDa); γ_T-2 (6kDa)	232-234
	One cloned	235-236
	2 seen on SDS-PAGE	237
	Antigenicity distinct from that of γ_Gs	238
	Silver staining distinct from that of γ_Gs	260
γ_G-a	(6 kDa), γ_G-b (10 kDa):	239
	Peptide map of 6 kDa form distinct from that of the 6 kDa γ_T	240
	Antigenicity distinct from that of γ_Ts	238
	One cloned	241
	At least two identified on SDS-PAGE	260
γ_G-c	(7 kDa):	231
	not cloned (?)	[b]
	Migration on SDS-PAGE distinct from that of other γ_Gs	260
	Antigenicity distinct from that of other γ_Gs	231

[a] β and γ subunits are purified as dimers composed of mixtures of the subunits described in this table.
[b] The protein of this cloned γ subunit has not yet been identified.

brane patches is not yet settled and, as shown later, is the object of intense research.[13-15] The recognition that G proteins may affect ionic channels under cell-free conditions led us, as well as others to investigate this possibility further. By the most recent count (August 1989), six classes of ionic channels, comprising at least 12 separate molecular species defined by distinct kinetic and pharmacological properties, have been shown to be stimulated or inhibited under cell-free conditions by exogenously added G protein α subunits or by activation of a nearby G protein by GTPγS, as seen in inside-out membrane patches or after incorporation of membrane vesicles into planar lipid bilayers. These ionic channels include channels that are essentially silent unless a G protein is activating them, referred to as G protein-gated ion channels,[2,12] as well as channels that are merely regulated by the G proteins, such as an ATP-sensitive, K^+ channel,[16-18] an amiloride sensitive monovalent cation channel,[19] a Ca^{2+}-activated, charybdotoxin-sensitive, K^+ channel,[20] and various voltage-gated ion channels,[4,5,22-24] (summarized in Table 5).

In addition to the approximately 12 channels thus far shown to be influenced by G proteins in a manner that appears to be direct, i.e., not involving protein phosphorylation or changes in levels of second messengers such as cyclic nucleotides, diacylglycerol, or Ca^{2+}, there may still be several more. This is suggested by reports on effects of PTX treatment or of GPTγS and G protein subunit injection on whole cell currents. These effects include the inhibition by GTPγS or pertussis toxin (PTX)-sensitive, G_o- and G_i-type G proteins of voltage-gated Ca^{2+} channels in chick[24] and rat[25-27] dorsal root ganglion cells and of what seem to be similar channels in AtT-20[28] and NG108-15[29] cells, and the stimulation by PTX-sensitive, G_i-type G protein of voltage-gated Ca^{2+} channels in adrenal Y1 and GH_3 cells[30,31] (summarized in Table 6). However, in these cases the involvement of soluble second messengers in the mediation of the effects of the G protein has yet to be ruled out.

The case of cell-free regulation of dihydropyridine (DHP)-sensitive, Ca^{2+} channels by G_s is of special interest. It was unexpected for two reasons: one because DHP-sensitive Ca^{2+} channels had been shown to be stimulated upon phosphorylation by the catalytic unit of cAMP-dependent protein kinase,[32] indicating that nature uses dual pathways to regulate a single function, one fast and membrane delimited, the other slower with a longer life span; the other, because it had been thought that G proteins might be "monogamous", i.e., specific for single effector functions, and here we were faced with proof for multifunctionality in G protein actions.

These advances were all the result of a multidisciplinary approach to the problem of signal transduction by G proteins which brought together classical biochemistry, sophisticated single channel recordings, and modern molecular biology. The background experiments, especially those of Nargeot et al.,[33] Soejima and Noma,[34] Pfaffinger et al.,[35] and Breitwieser and Szabo,[36] which led to the discovery that ionic channels are effector systems of G proteins akin to adenylyl cyclase and

Table 4
Classes G Protein Effector Functions

Effector class[a]	G Protein(s)[b]	Effect	Refs./Comment
	Proven in Cell-Free Assays		
Adenylyl cyclases	G_s/"G_i"	Stimulation/inhibition	Table 3
Retinal cGMP-specific PDE	G_t	Stimulation	Table 3
Phospholipase C	"G_p" (G_o?)	Stimulation	222,242,243
Phospholipase A_2	"G_p"	Stimulation	75,172,222
Ionic Channels (six types)	G_k = G_is/G_os; G_s	Stimulation/inhibition	Table 6
	Suggested by Indirect or Intact Cell Studies[c]		
Na/H Antiport	"G_p" (?)	Stimulation	244[d]
Voltage-gated Ca^{2+} channels	G_is, G_os (?)	Inhibition	Table 7
Insulin-sensitive glucose transporter	G_s (?)	Stimulation	245[e]
Liver Ca^{2+} Pump	G_s (?)	Inhibition	246[f]
Renal Na/K ATPase	?	Inhibition	247[g]
More ionic channels (?)	?	Stimulation/inhibition	Table 7
Phospholipase D	"G_p" (?)	Stimulation	76[h]
Nonretinal phosphodiesterases (PDEs)	?	Stimulation	248[i]

[a] The number of distinct gene products in each class may be high. For example, work from the laboratories of Gilman and Randall Reed shows that there are at least three adenylyl cyclases; the atrial, GH_3, and hippocampal neuron G_k-gated K^+ channels differ in conductance and G protein specificity; the heart and skeletal muscle dihydropyridine sensitive Ca^{2+} channels stimulated by G_s are products of separate genes.

[b] Except in the cases of adenylyl cyclase inhibition ("G_i") and phospholipase C activation ("G_p"), the G protein involved is known. In the cases of "G_i" and "G_p" *in vitro* experiments with activated purified α subunits have failed. Although some preliminary data indicate that the PTX sensitive "G_p" may be one of the G_o variants, confirmation is needed.

[c] These are systems in which mediation by phosphorylation has not been ruled out, but where indications are strong that a direct G protein regulation is possible.

[d] Inferred from persistent PTX-sensitive thrombin response in TPA-desensitized fibroblasts (Paris and Pouyssegur[244]).

[e] Inferred from kinetics and toxin sensitivity of agonist-mediated regulation of glucose transport in fat cells (Kuroda et al.[245]).

[f] Inferred from effect of glucagon peptide 19-29 on liver C pump (Mallat et al.[246]).

[g] Inferred from dopamine mediated regulation of Na-K ATPase (Bertorello and Aperia[247]).

[h] Inferred from the fact that receptors that increase phospholipase D activity are coupled by G proteins and that GTPγS stimulates phospholipase D activity in isolated membranes (Bocckino et al.[76]).

[i] Inferred from agonist-induced PTX-insensitive increased rates of cAMP hydrolysis in Hughes et al.[248]

Table 5
Ionic Channels Regulated Under Cell-Free Conditions by Pure G Protein and/or GTPγS

Channel type	G protein	Effect	Tissue/cell	Refs.
K_{Gk} 40 pS	G_i (1,2,3)	Stim.	Heart	2,7,8,10,96
K_{Gk} 50 pS	G_{i3}	Stim.	GH_3	3,8,43
K_{Gk} 4 types	G_{o1}	Stim.	Hippocampal neurons	12
K_{ATP}	G_{i3}	Stim.	RIN, heart, skeletal muscle	16-18
$Na(K)_{[Amil]}$	G_{i3}	Stim.	Renal med. collect. tubule	19
$Ca_{[DHP]}$L-type	G_s (all 4)	Stim.	Heart, skeletal muscle	4,5,11,21
$Na_{[TTX]}$	G_s	Inh.	Heart	22
$K_{Ca[Charyb]}$	? (Iso/GTP)	Stim.	Uterine smooth muscle	20
$Ca_{[Ni]}$T-type	? (GTPγS)	Inh.	Rat DRGs	23

Table 6
Ionic Channels That May Be Under Direct Regulation by G Proteins as Inferred from Whole Cell Recordings

Channel type	Agonist	G protein*	Effect	Cell	Mimicked by TPA	Refs.
Ca (N-type?)	Opioid	$G_o > G_i$	Inh.	NG108-15	?	29
Ca (L-type)	Ang II	G_i	Stim.	Y1 Adrenal	?	30
Ca (L-type)	GnRH	G_i/G_o	Stim.	GH_3	?	31
Ca (L-type)	SST	? GTPγS	Inh.	AtT-20	Yes	28
Ca (?)	$GABA_{(B)}$	? GTPγS	Inh.	Chick DRG	Yes (total)	24,249
	NE (αAR)	? GTPγS	Inh.	Chick DRG	Yes	24,249
Ca (?)	$GABA_{(B)}$	? GTPγS	Inh.	Rat DRG	No	25,250,251
Ca (?)	NPY	$G_o > G_i$	Inh.	Rat DRG	Yes (partial)	26,252
	Bradyk.	$G_o = G_i$	Inh.	Rat DRG	Yes	27

* All the G proteins involved are PTX-sensitive.

cGMP-phosphodiesterase, and the initial experiments showing effects of purified G proteins and protein subunits on channels in excised membrane patches, were reviewed in Brown and Birnbaumer.[1] The present article focuses on some of the more recent results from our laboratories dealing with a PTX-sensitive, G_i-type family of G proteins, their effects of ionic channels, our efforts in assigning defined functions to individual G proteins as they are known from biochemical and molecular cloning studies, and some speculations that follow from the results obtained as to why G proteins dissociate and how transduction pathways are set up.

II. PRIMARY STRUCTURE OF G PROTEINS

The primary αβγ structure of G proteins is summarized in Figure 2 and Tables 2 and 3, and has been reviewed by Lochrie and Simon,[37] as well as by us.[38] Key to the understanding of their functioning is of course both their subunit dissociation cycle superimposed on their GTPase cycle, and their transient — or perhaps not so transient — interactions with receptors and effectors. In our view it is clear that the receptor signal is carried to the effector by the α subunit as exemplified in the experiment of Figure 3. At the time of this writing, 12 α subunits, encoded in 9 genes, 4 β subunits, and at least 3 γ subunits (all mammalian) are known (Tables 2 and 3). β and γ subunits form dimers which may be of several types, $\beta_{36\gamma}$ and $\beta_{35\gamma}$, if a cell expresses only one type of γ subunit, or of more types if it expresses more β or γ subunits. To our knowledge, α subunits, when combining with βγ dimers to form holo-G proteins, do not distinguish among βγ dimers (Figure 4). This is not to say that all tissues have the same complement of βγ dimers. Quite the contrary, as shown in Figure 5, β_{35}/β_{36} ratios differ in human placenta, human erythrocytes, bovine brain, and bovine retinal rod cells. α Subunits bind GTP, hydrolyze GTP, dissociate from the βγ dimer on activation by GTP analogs, and/or $NaAlF_4$/GDP, and, with few exceptions, are substrates for ADP-ribosylation by cholera and/or pertussis toxin (CTX and PTX). Studies with transducin-α identified an arginine at the approximate center of the molecule as the amino acid ADP ribosylated by CTX[39] and a cysteine at position −4 from the carboxyl terminal end as the site of ADP-ribosylation by PTX.[40] It is worth mentioning that not all G proteins have been either purified or cloned, and that not all the known G proteins, such as G_{o1} and G_{o2}[41,42] or some of the G_is, have unequivocally assigned functions, or that all functionally recognized G proteins, such as the inhibitory G_i of adenylyl cyclase or the stimulatory G_p of phospholipases, have been identified.

III. FUNCTIONAL STUDIES

A. Combined Use of Natural and Recombinant α Subunits Made in Bacteria to Define Their Functions

The abundance at which different α_s and α_i molecules are expressed varies from tissue to tissue, raising the question as to whether functional differences are associated with the structural differences or whether the G_ss and, respectively, the G_is should be thought of merely as isoforms. Although the final word on these questions is not yet in, we have during this last year developed the method(s) required to answer them. Thus, we have so far succeeded in purifying two types of G_i from human erythrocytes (hRBCs) and a third from bovine brain, from which we also purified the two forms of G_o. This allowed us to test for potential differential biological functions. We also expressed biologically active forms of the α subunits in bacteria, designated as recombinant α subunits, so that we could predict/confirm biological functions of various cloned and or purified G protein α subunits.

We have not yet carried out all of the studies. However, we were able to determine first of all that both the natural purified and the recombinant forms of α_{i3}, α_{i1}, and α_{i2} all stimulate K^+

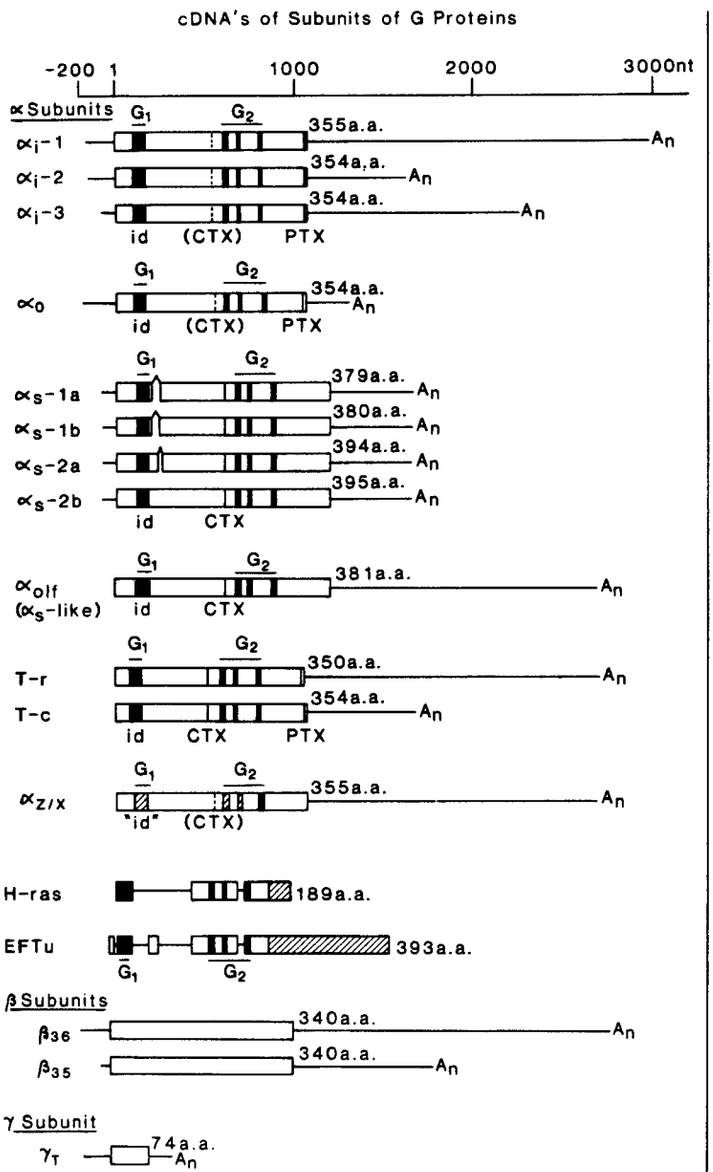


FIGURE 2. Schematic representation of vertebrate α subunit mRNA molecules as deduced from cDNA cloning. Open boxes represent the open reading frames or coding sequences and lines represent 5′ and 3′ untranslated sequences which may be incomplete. Black boxes within the open reading frames of α subunits denote sequences highly homologous to those known in bacterial elongation factor TU to be involved in GTP binding and hydrolysis. Sequences homologous to these are present also in the *ras* molecules. The mRNA molecules encoding the β_{36}, β_{35}, and γ_T are shown for comparison. The position of amino acids ADP-ribosylated by CTX and PTX are indicated. i.d., location of the identity box. The scale is in nucleotides.

channels (Figure 6; References 7 and 43) We failed to observe significant differences in the potency with which type 1, 2, and 3 α_i molecules activated K^+ channels, even though the recombinant forms all had potencies between 30- and 50-fold lower than their natural counterparts.[7] Thus, with respect to

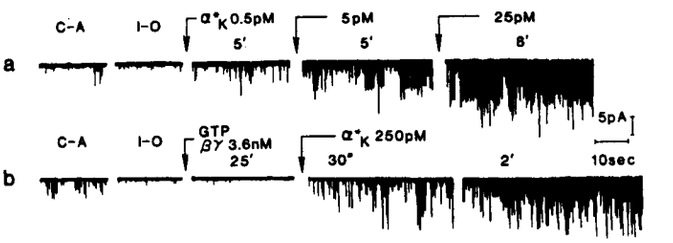


FIGURE 3. (a) Stimulation of single channel K^+ currents in GH_3 cell membrane patches by increasing concentrations of native GTPγS-activated human erythrocyte α_{i3} (α_k^*). (b) Lack of intrinsic stimulatory effects of human erythrocyte βγ dimer added in the presence of GTP in a responsive membrane patch. Lubrol PX (500 n*M*) was present throughout. This and similar figures shown next present segments of records obtained in the cell-attached mode (C-A) and in the inside-out mode after patch excision and before additions (I-O). Additions are denoted by arrows and time elapsed between addition of test substances are given above the records.[9]

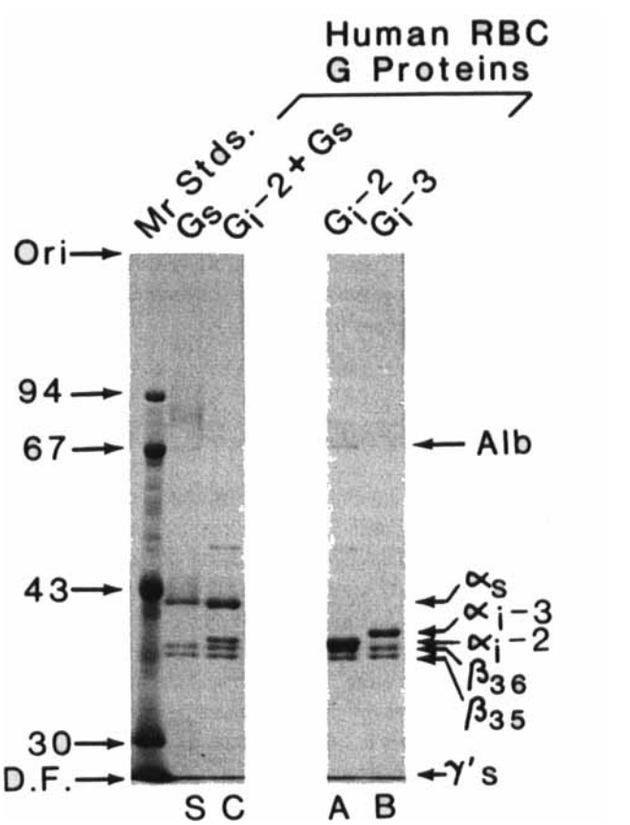


FIGURE 4. Urea gradient/SDS-PAGE analysis of G_s, G_{i-2}, and G_{i-3} purified from human erythrocyte membranes. Shown is the photograph of a Coomassie blue stained gel. Note: (1) differential migration of α subunits as well as of the two β subunits, (2) that each of the proteins has its share of βγ dimers, and (3) that this does not differ from one-to-another.

atrial muscarinic K^+ channels, G_{i1}, G_{i2}, and G_{i3} must be considered iso-G proteins. Studies are currently in progress in atrial membrane patches in which the endogenous G_k has been

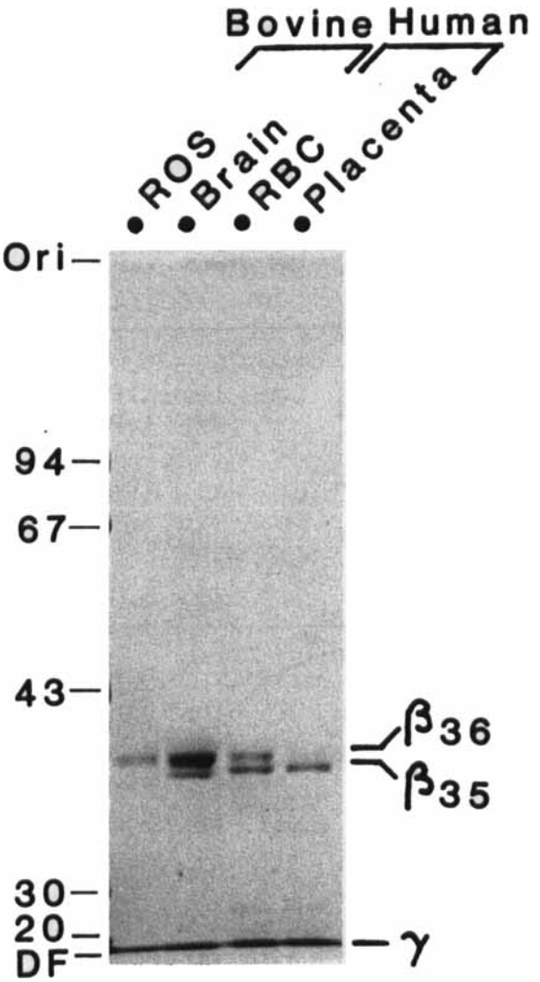


FIGURE 5. Urea gradient/SDS-PAGE analysis of four preparations of βγ dimers. Shown is the photograph of a Coomassie blue stained gel. Note that different tissues express β_{35} and β_{36} at different ratios.

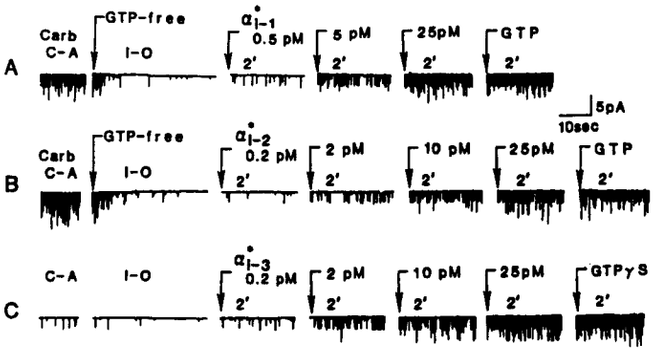


FIGURE 6. Lack of significant difference in stimulation of atrial G_k-gated K^+ channels obtained with the activated α subunits of G_{i-1}, G_{i-2}, and G_{i-3}. Presence or absence carbachol (Carb) is denoted at the beginning of each train of records.[7]

uncoupled from receptors by treatment with PTX,[1,3] to determine whether muscarinic and/or P_2 purinergic receptors exhibit selectivity for interaction with one or another of the G_i proteins.

Second, by testing the effect of recombinant α_s, we were able to determine that the stimulation of Ca^{2+} channels obtained with purified hRBC G_s[4,5,11] is indeed mediated by G_s as opposed to being due to a contaminant. Furthermore, in collaboration with Michael Graziano and Al Gilman we tested the recombinant forms of three of the possible four splice variants of α_s for their Ca^{2+} channel stimulatory activity and found that they all do so with indistinguishable potency and efficacy.[21] As was the case with recombinant α_i subunits, the recombinant α_s subunits also displayed a 20-fold reduced potency with respect to that of native human erythrocyte α_s. This applied not only to Ca^{2+} channel stimulation, but equally to adenylyl cyclase stimulation. Very likely, bacteria fail to carry out a critical posttranslational modification that exists only in eukaryotic cells, or alternatively, modify the α subunit in a manner that eukaryotic cells do not.

B. Gating of Ionic Channels as a Tool to Discover New Roles for G Proteins: Effects of G_o on Neuronal K^+ Channels

One of the properties of the "muscarinic" K^+ channels is that they are essentially silent in the absence of stimulation by an activated G protein (G_k). That is, in the absence of activated G protein their Po is close to zero. The possibility existed that not only G_i proteins regulate K^+ channels but also the structurally closely related G_o. Since nervous tissue is rich in G_o, central nervous system neurons, specifically hippocampal pyramidal cells, were placed into culture and studied for potential presence of both G_i- and G_o-gated K^+ channels. Although these studies are still in progress,[258] the initial findings with highly purified bovine brain GTPγS-activated G_{o1} (G^*_{o1}) were of interest.[12] They identified the existence of several novel G-protein gated, more precisely G_o-gated K^+ channels, that are distinct from G_i-gated K^+ channels. Thus, application of purified bovine brain G^*_{o1} to the cytoplasmic aspect of inside-out membrane patches of cultured hippocampal pyramidal cells resulted in appearance of three new types of single channel K^+ currents consistent with the existence of the three nonrectifying of K^+ channels having sizes of 13, 40, and 55 pS, respectively, plus an inwardly rectifying K^+ channel with a slope conductance of 40 pS. No such channel activities were observed with hRBC $G^*_i - 3$ or hRBC $\alpha^*_i - 3$. G^*_{o2} or α^*_{o2} have not been tested in this system as yet. In contrast to earlier observations with G^*_o added to guinea pig atrial membrane patches, which showed only marginal effects of GTPγS-activated G_{o1} at 2 n*M*[2], the hippocampal K^+ channel is highly sensitive to G^*_{o1},[12] and significant activation was obtained at 1 p*M* and half maximal effects were obtained at about 10 p*M*. The identity of the active G protein in the G_{o1} preparations used was confirmed with recombinant GTPγS-activated α_{o1}. The G_o-gated channels were

stimulated in the absence of Ca^{2+} or ATP, in the presence or absence of AMP-P(NH)P, added routinely to inhibit ATP-sensitive, 70 pS K^+ channels. Furthermore, EGTA did not interfere with the actions of G_{o1} or *recombinant* α_{o1}. Thus, in hippocampal pyramidal cells of the rat, G_{o1} is a G_k, and the K^+ channels gated by it are several and differ from those present in atrial cells in various aspects including G protein specificity. These findings raise the question which if any of the G proteins that gate K^+ channels regulate the other known PTX sensitive effector systems.

IV. βγ DIMERS INHIBIT K^+ CHANNEL GATING BY G PROTEIN

Logothetis et al.[13,14] reported twice that βγ dimers stimulate atrial K^+ channel activity. Their finding is not reproduced in our hands. Quite the contrary, when we add βγ dimers to inside-out membrane patches in which K^+ channels have been stimulated either by GTP only (baseline activity) or by carbachol plus GTP (agonist-stimulated activity), we find consistently inhibition of activity (Figure 7). On their own, i.e., when added to silent patches in the absence of GTP, βγ dimers have no effect under our assay conditions (Figure 3; References 8 to 10).

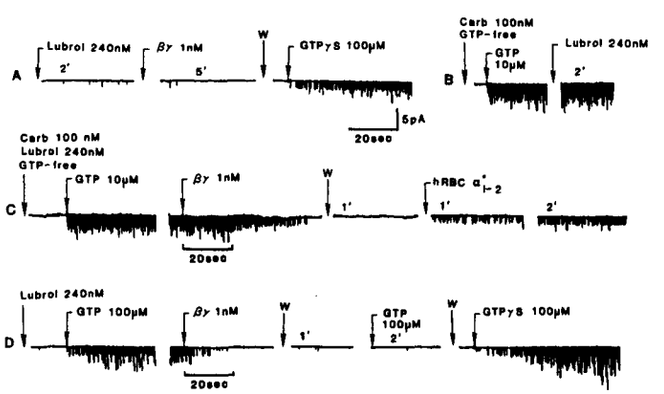


FIGURE 7. Inhibition of G protein-gated K^+ channel activity by βγ dimers. Inside-out membrane patches from adult guinea pig atrial cells were exposed to the bathing solution (140 m*M* KCl, 2 m*M* $MgCl_2$, 5 m*M* EGTA, 10 m*M* Hepes-K, pH 7.4) containing the additives shown on the figure., The pipette solution was identical to the bathing solution and contained 100 n*M* carbachol when indicated. The composition of bathing solution was changed by a concentration clamp method. Note that Lubrol PX and/or bovine serum albumin, used to maintain βγ dimers in suspension do not interfere with stimulation of activity by GTP (D) or GTP plus agonist (B, C), and that βγ dimers have no effect on their own (A) but inhibit atrial K^+ channel stimulation by the membrane G_k (C, D). Inhibition is faster and is elicited with lower concentrations of βγ dimers when K^+ channels are operating under baseline conditions (GTP only, experiment D: cessation of activity after 16 s) than when they are stimulated by agonist (Carbachol plus GTP: experiment C: cessation of activity after 50 s). Numbers above records denote time elapsed in min between solution change and the beginning of the record shown.

Concentration effect studies showed clearly that βγ dimers are more potent in inhibiting agonist-independent than agonist-stimulated activity and that this phenomenon applies not only to βγ dimers suspended in Lubrol-PX, such as those from human placenta, human erythrocytes, and bovine brain, but also to βγ dimers presented to the patches in aqueous media, such as transducin βγ (Figure 8).

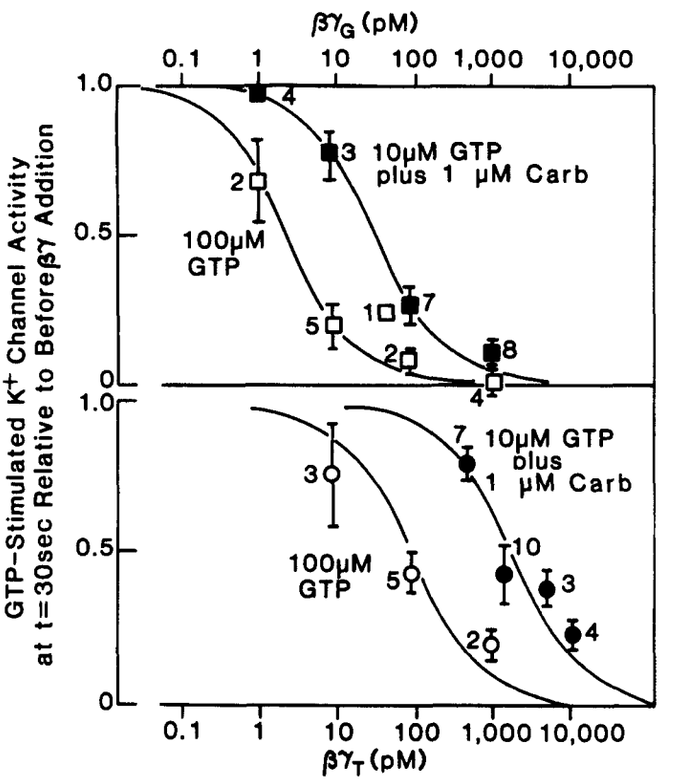


FIGURE 8. Effect of agonist (carbachol) on the dose-dependent inhibition by βγ dimers of GTP-dependent K^+ channel activities in inside-out guinea pig atrial membrane patches by βγ dimers. When 1 μ*M* carbachol was present in the pipette, GTP was 10 μ*M*; in the absence of carbachol GTP was 100 μ*M*. βγG: data obtained with βγ dimers derived from either human erythrocyte, human placenta, or bovine brain were pooled. $\beta\gamma_t$: data obtained with βγ derived from bovine rod outer segments. (We thank Dr. Tony Evans for the gift of human placental βγ dimers, and Dr. J.-K. Ho, for the gift of bovine rod outer segment βγ dimers.)

The fact that βγ dimers inhibit GTP-dependent activity in the absence of agonist at lower concentrations as compared to inhibition in the presence of agonist was observed previously in purified components reconstituted into phospholipid vesicles (Figure 9; References 44 and 45) and serves to support our thesis that βγ dimers act in intact membranes as suppressor of "noise" generated by agonist-unoccupied receptors.

The inhibitory effects of βγ dimers obtained by us need to be contrasted to stimulatory effects obtained by Clapham, Neer, and their collaborators.[13-15,46] We do not understand exactly

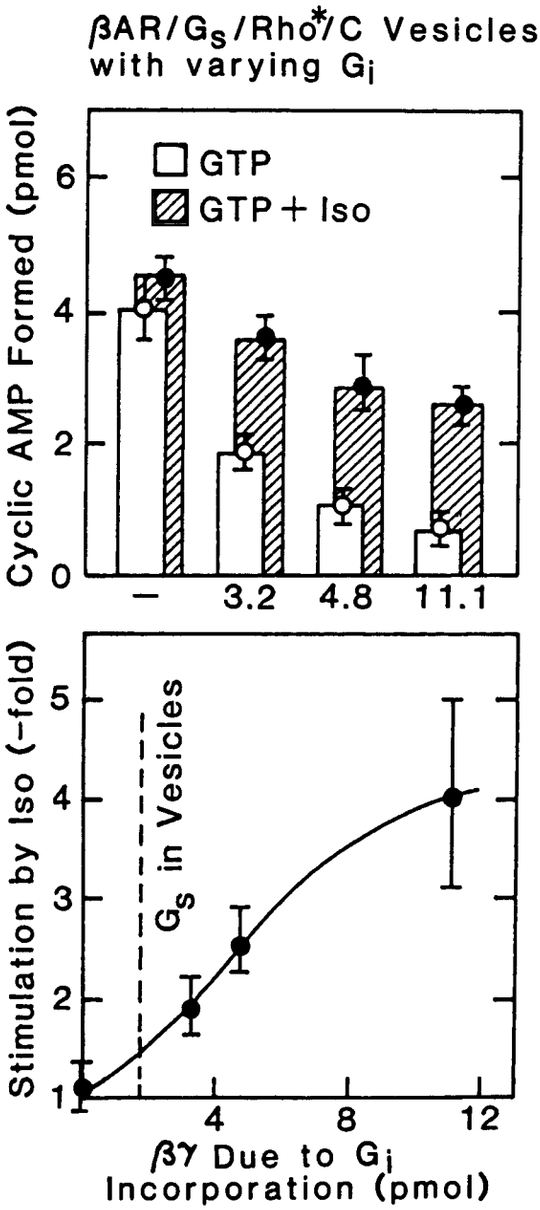


FIGURE 9. βγ Dimers formed upon activation of G_i by rhodopsin, are potent inhibitors of agonist-independent but not of agonist-stimulated G_s activity. β-Adrenoceptors, G_s and resolved bovine brain catalytic unit of adenylyl cyclase were incorporated into phospholipid vesicles in the presence of photoactivated rhodopsin and the indicated amounts of G_i. Vesicles were resuspended in buffer with GTP and assayed for adenylyl cyclase activity in the absence and the presence of the β-adrenoceptor agonist isoproterenol. Top panel: absolute activities; Bottom panel: relative stimulation by isoproterenol. Note that the selective inhibition of receptor plus GTP induced activation of G_s by βγ dimer is an essential feature for functional expression of the agonist effect.[44]

the reasons for the discrepancy since we obtain inhibition also in the absence of Lubrol PX using βγ dimers from transducin which are water soluble. The claim that βγ dimers may be acting by stimulation of arachidonic acid formation and acting through a metabolite[46] is suspect. In an adjacent report, Kurachi et al.[47] describe that arachidonic acid and its metabolites require for their action the presence of GTP and are blocked by GDPβS. Logothetis et al.[13,15] and presumably also Kim et al.[46] obtain stimulation with βγ dimers in the absence of GTP.

V. CURRENT VIEWS ON HOW SIGNAL TRANSDUCTION BY GTP AND RECEPTORS COMES ABOUT AND WHICH RECEPTOR ACTS ON WHICH G PROTEIN TO REGULATE WHICH EFFECTOR SYSTEM

Taken together the results discussed in the previous sections lead to several conclusions: (1) ionic channels are targets of direct regulation by G proteins as are adenylyl cyclase and the cGMP-specific phosphodiesterase; (2) α subunits and not βγ dimers are the specificity determinants of signal transduction pathways; (3) several G proteins may have the same function, e.g., stimulation of K^+ channels by three G_is; and (4) a single G protein may have more than one function, i.e., be multifunctional (e.g., stimulation of adenylyl cyclase and the dihydropyridine sensitive Ca^{2+} channel by a single G_s).

Two independent sets of questions emerge from these findings. The first deals with the subunit dissociation reaction and asks what type of advantage it confers onto the system by its existence. An answer to this can be found upon analyzing in detail the G protein regulatory cycle and the mechanism by which receptors promote G protein activation by GTP.

The second set of questions deals with crosstalk between signal transduction pathways, i.e., whether receptors act on more than one G protein, and if so, which and whether G proteins interact with more than one receptor as well as with more than one effector, and if so, how frequent this is.

A. Role of Subunit Dissociation: Requirement for Catalytic Action of Receptors

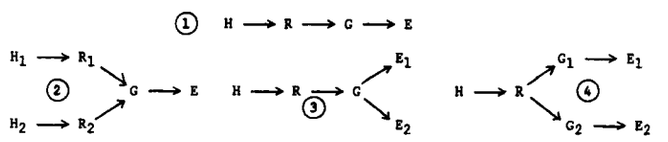
In the second half of the 1970s, it was demonstrated that receptors act catalytically rather than stoichiometrically to activate adenylyl cyclase,[48] and in 1980 it was found that receptors in addition to promoting GDP/GTP exchange[49,50] also participate in promoting the activation reaction proper of adenylyl cyclase by GTP and its analogs,[51-53] reviewed in Reference 54. These two findings, catalytic action and stabilization of the nucleotide-activated form of adenylyl cyclase, now known to be in fact G_s, are thermodynamically impossible, unless the stabilized form of the G protein undergoes some type of additional spontaneous change that releases it from

microscopic reversibility constraints, which happens in all enzymatically catalyzed reactions where the product is chemically different from the starting substrate. Dissociation of βγ from the activated and stable receptor-G protein complex is such a reaction change. We therefore propose that the role of the dissociation reaction is a requirement without which receptors cannot act catalytically. Both the catalytic nature of receptor-mediated activation of G_s and the fact that receptor affects not only GDP release but also the rate at which inactive guanine nucleotide-occupied G protein isomerizes from an inactive to an active state have been confirmed in reconstitution experiments with purified receptor and purified G_s.[55,56] Since receptors do not interact with the α subunits except in the context of βγ,[57-61] reassociation of the GTPase-deactivated α with βγ is essential for restimulation by receptors. This then leads to a description of the G protein regulatory cycle under influence of receptor as depicted in Figure 10.

Taken together, it is thus possible to ascribe three generic functions to βγ dimers (Table 7): (1) activation of α subunit by receptor, for without βγ, receptors do not interact with α; (2) amplification of the receptor signal, for without dissociation, receptors cannot act catalytically; and (3) noise reduction, for unoccupied receptors are not silent.

B. Specificities in Receptor-G Protein-Effector Interaction

Ever since the discoveries in the late 1960s that up to five different hormone receptors can activate a single adenylyl cyclase system in an isolated membrane[62,63] and, in the early and mid-1970s, that receptors can be transferred from one cell to another[64] and that there are no species and/or tissue specificity restrictions as to the source of G_s for reconstitution of a hormonally stimulable adenylyl cyclase system in *cyc*$^-$ membranes,[65,66] it has been clear that single G proteins are designed to interact with classes of receptors as opposed to single receptor subtypes. The discovery that the same splice variant of G_s that activates adenylyl cyclase is also able to regulate Ca^{2+} channel activity[21] indicates that one G protein can interact with more than one effector. The discovery that three different G_i proteins all activate the same K^+ channel,[7] indicates that several G proteins may regulate a single effector. Ashkenazi et al.[67] showed that single receptors may affect more than one G protein. It follows that transmembrane wiring diagrams are in fact combinations of the following four basic configurations:



The complexity that may exist in the wiring of transmembrane signal transmission was well illustrated by the findings of Ewald et al.[27] in PTX-treated rat sensory neurons. On studying the efficacy with which brain G_i and G_o reconstitue Ca^{2+} current regulation by neuropeptide Y (NPY) and bradykinin, they discovered that the effect of NPY could be fully reconstituted with G_o, with G_i being much less potent, while the effect of

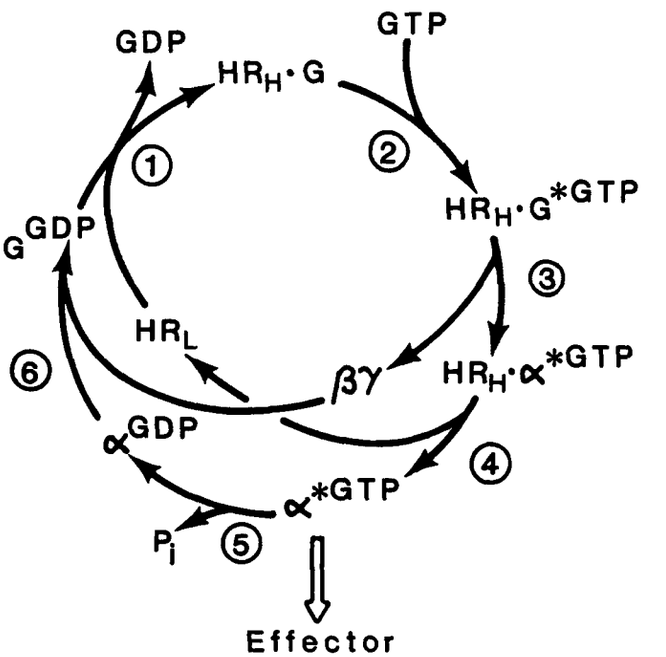
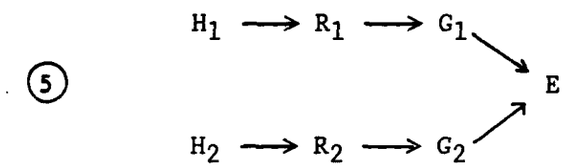


FIGURE 10. Integrated view of receptor-mediated catalytic activation of a G protein in the context of the dual subunit dissociation and GTPase cycles of G proteins. The role of receptors is to promote nucleotide exchange and to stabilize a GTP-dependent "activated" form of the G protein and the G protein undergoes a cyclical dissociation-reassociation reaction and oscillates between GDP, nucleotide free, and GTP states. The cycles are driven energetically forward by the capacity of the G protein to hydrolyze GTP[253-257] and kinetically by the dissociation of βγ dimer from the activated receptor-G proteins complex. The receptor has high affinity for agonist (R_H) when associated with the nucleotide-free trimeric αβγ form of the G protein, and has low affinity for the agonist (R_L) when it is free. Furthermore, the receptor has higher affinity for the trimeric αβγ form of G than the G-GDP thus accounting for the finding that GDP and GDP analogs promote the R_H to R_L transition. The βγ dimers are required for the interaction of α with R, and after formation of G-GTP, no G* forms unless it is "aided" by receptor. Thus, receptor has an even higher affinity for the G*GTP state than the nucleotide free state of G. As a consequence receptor dissociation is absolutely dependent on reaction 2 (subunit dissociation). Thermodynamic reasons do not allow R both to stabilize the G* state and to dissociate from it. Reaction 3 states further that the α*GTP loses its ability to stay associated with receptor and decomposes further into free activated α*GTP plus free receptor, thus accounting for the fact that under "working" conditions (saturation by both GTP and hormone, and hence sustained regulation of effector) only a small proportion of receptors are found in their high affinity, G-protein associated state. It follows that the G protein cycle is driven forward not only by the GTPase but also, and obligatorily so, by the subunit dissociation reaction. The scheme accounts for the experimental findings: (a) that receptors act catalytically and therefore need to dissociate from the G protein at one time or another;[48] and (b) that receptors accelerate the transition from inactive G-GTPγS to active G*-GTPγS transition and therefore must have higher intrinsic affinity for the activated than the inactive state.[51-53]

Table 7
Intramembrane Roles of G Protein βγ Dimers

Reaction	Product	Role
Reassociation with α^{GDP}	G^{GDP}	Activation by R
Dissociation from HR.G*GTP	HR.α*GTP	Signal amplification
Reassociation with R.α*GTP	R.G^{GTP}	Noise reduction

bradykinin could only be partially reconstituted by G_o, requiring G_i to achieve full reconstitution (Diagram 5).



The important notion that emerges from these findings is that the wiring diagrams describing signal transduction by G proteins needs to be determined individually and separately for each cell or tissue of interest. This includes the determination not only of which receptors are present but also which G proteins and effectors process the receptor signals.

VI. CONCLUSION

Signal transduction by G proteins is a fundamental and widespread mechanism used by a wide variety of hormones, neurotransmitters, and auto- and paracrine factors to regulate cellular functions. G proteins modulate not only cAMP formation, but also intracellular Ca^{2+} mobilization, arachidonic acid release, and, very importantly, membrane potential, with the latter not only being a trigger for neurotransmitter release, but also a conductor of nerve impulses. In tissues such as secretory cells, it is the main regulator of Ca^{2+} entry. In heart, action potentials play the dual role of determining the frequency of contraction, and through modulation of the duration of the depolarized state, membrane potential determines Ca^{2+} entry and the force of contraction. More subtle changes in resting membrane potential alter the cell's predisposition to be stimulated by other factors and hormones. It is easy to imagine that persistent changes in membrane potential may affect acutely and chronically the cell's proliferative properties.

The mechanism by which G proteins are activated provides for amplification, reversal of action, and continued monitoring of hormone: for an amplification because few receptor molecules may act catalytically to activate many G proteins molecules; for reversal of action because they have an internal turnoff mechanism whereby the Gα subunit hydrolyzes GTP to GDP; and for continued monitoring of the primary messenger level because each activation cycle requires not only GTP but also occupied receptor.

Not all G proteins are known and some are known of which their functions are still unknown. However, more G proteins and more effector functions affected by them will surely be found. Work is in progress to unravel a complicated network of interactions between receptors, G proteins, and effector systems that not only affect regulation of metabolic activities of organs such as liver, heart, and fat, but also of the integrative functions of the central nervous system.

ACKNOWLEDGMENTS

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REFERENCES

1. **Brown, A. M. and Birnbaumer, L.,** Direct G protein gating of ion channels, *Am. J. Physiol.*, 23, H401, 1988.
2. **Yatani, A., Codina, J., Brown, A. M., and Birnbaumer, L.,** Direct activation of mammalian atrial muscarinic potassium channels by GTP regulatory protein G_k, *Science*, 235, 207, 1987.
3. **Yatani, A., Codina, J., Sekura, R. D., Birnbaumer, L., and Brown, A. M.,** Reconstitution of somatostatin and muscarinic receptor mediated stimulation of K^+ channels by isolated G_k protein in clonal rat anterior pituitary cell membranes, *Mol. Endocrinol.*, 1, 283, 1987.
4. **Yatani, A., Codina, J., Imoto, Y., Reeves, J. P., Birnbaumer, L., and Brown, A. M.,** A G protein directly regulates mammalian cardiac calcium channels, *Science*, 238, 1288, 1987c.
5. **Yatani, A., Imoto, Y., Codina, J., Hamilton, S. L., Brown, A. M., and Birnbaumer, L.,** The stimulatory G protein of adenylyl cyclase, G_s, directly stimulates dihydropyridine-sensitive skeletal muscle Ca^{2+} channels. Evidence for direct regulation independent of phosphorylation by cAMP-dependent protein kinase, *J. Biol. Chem.*, 263, 9887, 1988.
6. **Yatani, A., Hamm, H., Codina, J., Mazzoni, M. R., Birnbaumer, L., and Brown, A. M.,** A monoclonal antibody to the α subunit of G_k blocks muscarinic activation of atrial K^+ channels, *Science*, 241, 828, 1988.
7. **Yatani, A., Mattera, R., Codina, J., Graf, R., Okabe, K., Padrell, E., Iyengar, R., Brown, A. M., and Birnbaumer, L.,** The G protein-gated atrial K^+ channel is stimulated by three distinct $G_{i\alpha}$-subunits, *Nature*, 336, 680, 1988c.
8. **Codina, J., Yatani, A., Grenet, D., Brown, A. M., and Birnbaumer, L.,** The *alpha* subunit of the GTP binding protein G_k opens atrial potassium channels, *Science*, 236, 442, 1987.
9. **Codina, J., Grenet, D., Yatani, A., Birnbaumer, L., and Brown, A. M.,** Hormonal regulation of pituitary GH_3 cell K^+ channels by G_k is mediated by its *alpha* subunit, *FEBS Lett.*, 216, 104, 1987b.
10. **Kirsch, G., Yatani, A., Codina, J., Birnbaumer, L., and Brown, A. M.,** The *alpha* subunit of G_k activates atrial K^+ channels of chick, rat and guinea pig, *Am. J. Physiol.*, 254 (*Heart Circ. Physiol.*, 23), H1200, 1988.
11. **Imoto, Y., Yatani, A., Reeves, J. P., Codina, J., Birnbaumer, L.,**

and Brown, A. M., α subunit of G_s directly activates cardiac calcium channels in lipid bilayers, *Am. J. Physiol.*, 255, H722, 1988.

12. **VanDongen, A., Codina, J., Olate, J., Mattera, R., Joho, R., Birnbaumer, L., and Brown, A. M.,** Newly identified brain potassium channels gated by the guanine nucleotide binding (G) protein G_o, *Science*, 242, 1433, 1988.
13. **Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., and Clapham, D. E.,** The βγ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart, *Nature*, 325, 321, 1987.
14. **Kurachi, Y., Ito, H., Sugimoto, T., Katada, T., and Ui, M.,** Activation of atrial muscarinic K^+ channels by low concentrations of βγ subunits of G rat brain protein, *Pflügers Arch.*, 413, 325, 1989a.
15. **Logothetis, D. E., Kim, D., Northup, J. K., Neer, E. J., and Clapham, D. E.,** Specificity of action of guanine nucleotide-binding regulatory protein subunits on the cardiac muscarinic K^+ channel, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 5814, 1988.
16. **Parent, L. and Coronado, R.,** Reconstitution of the ATP-sensitive potassium channel of skeletal muscle. Activation of a G-protein dependent process, *J. Gen. Physiol.*, in press, 1989.
17. **Ribalet, B., Ciani, S., and Eddlestone, G. T.,** Modulation of ATP-sensitive K channels in RINm5F cells by phosphorylation and G proteins, *Biophys. J.*, 55, 587A, 1989.
18. **Kirsch, G., Codina, J., Birnbaumer, L., and Brown, A. M.,** G protein regulation of the ventricular ATP-sensitive K^+ channel (unpublished).
19. **Light, D. B., Ausiello, D., and Stanton, B. A.,** A GTP binding protein, $\alpha_i^* - 3$, directly activates a sodium-conducting ion channel in a renal epithelium, *J. Clin. Invest.*, in press, 1989.
20. **Toro, L., Ramos-Franco, J., and Stephani, E.,** K_{Ca} channels in myometrium can be directly activated by a G-protein coupled to a β-adrenergic receptor, submitted, 1989.
21. **Mattera, R., Graziano, M. P., Yatani, A., Zhou, Z., Graf, R., Codina, J., Birnbaumer, L., Gilman, A. G., and Brown, A. M.,** Individual splice variants of the α subunit of the G protein G_s activate both adenylyl cyclase and Ca^{2+} channels, *Science*, 243, 804, 1989.
22. **Schubert, B., VanDongen, A. M. J., Kirsch, G. E., and Brown, A. M.,** Modulation of cardiac Na channels by beta-adreno receptors and the G protein G_s, *Biophys. J.*, 55, 229A, 1989.
23. **Dolphin, A. C., Scott, R. H., and Wooton, J. F.,** Photorelease of GTPγS inhibits the low threshold channel current in cultured rat dorsal root ganglion (GRG) neurons, *Pflügers Arch.*, 411, 19P, 1988.
24. **Holz, G. G., Rane, S. G., and Dunlap, K.,** GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels, *Nature*, 319, 670, 1986.
25. **Scott, R. H. and Dolphin, A. C.,** Regulation of calcium currents by GTP analogue: potentiation of (−)-baclofen-mediated inhibition, *Neurosci. Lett.*, 69, 59, 1986.
26. **Ewald, D. A., Sternweis, P. C., and Miller, R. J.,** Guanine nucleotide-binding protein G_o-induced coupling of neuropeptide Y receptors to Ca^{2+} channels in sensory neurons, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 3633, 1988.
27. **Ewald, D. A., Pang, I.-H., Sternweis, P. C., and Miller, R. J.,** Differential G protein-mediated coupling of neurotransmitter receptors to Ca^{2+} channels in rat dorsal root ganglion neurons in vitro, *Neuron*, 2, 1185, 1989.
28. **Lewis, D. L., Weight, F. F., and Luini, A.,** A guanine nucleotide-binding protein mediates the inhibition of voltage-dependent calcium current by somatostatin in a pituitary cell line, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 9035, 1986.
29. **Hescheler, J., Rosenthal, W., Trautwein, W., and Schultz, G.,** The GTP-binding protein, N_o, regulates neuronal calcium channels, *Nature*, 325, 445, 1987.
30. **Hescheler, J., Rosenthal, W., Hinsch, K.-D., Wulfern, M., Trautwein, W., and Schultz, G.,** Angiotensin II-induced stimulation of voltage-dependent Ca^{2+} currents in an adrenal cortical cell line, *EMBO J.*, 7, 619, 1988.
31. **Rosenthal, W., Hescheler, J., Hinsch, K.-D., Spicher, K., Trautwein, W., and Schultz, G.,** Cyclic AMP-independent, dual regulation of voltage-dependent Ca^{2+} currents by LHRH and somatostatin in a pituitary cell line, *EMBO J.*, 7, 1627, 1988.
32. **Kameyama, M., Hescheler, J., Hofmann, F., and Trautwein, W.,** Modulation of Ca current during the phosphorylation cycle in the guinea pig heart, *Pflügers Arch.*, 407, 121, 1986.
33. **Nargeot, J., Nerbonne, J. M., Engels, J., and Lester, H. A.,** Time course of the increase in the myocardial slow inward current after a photochemically generated concentration jump of intracellular cAMP, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 2395, 1983.
34. **Soejima, M. and Noma, A.,** Mode of regulation of the ACh-sensitive K-channel by the muscarinic receptor in rabbit atrial cells, *Pflügers Arch.*, 400, 424, 1984.
35. **Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathanson, N. M., and Hille, B.,** GTP-binding proteins couple cardiac muscarinic receptors to a K channel, *Nature*, 317, 536, 1985.
36. **Breitwieser, G. E. and Szabo, G.,** Uncoupling of cardiac muscarinic and beta-adrenergic receptors from ion channels by a guanine nucleotide analogue, *Nature*, 317, 538, 1985.
37. **Lochrie, M. A. and Simon, M. I.,** G protein multiplicity in eukaryotic signal transduction systems, *Biochemistry*, 17, 4957, 1988.
38. **Birnbaumer, L., Codina, J., Yatani, A., Mattera, R., Graf, R., Olate, J., Themmen, A. P. N., Liao, C.-F., Sanford, J., Okabe, K., Imoto, Y., Zhou, Z., Abramowitz, J., Suki, W. S., Hamm, H. E., Iyengar, R., Birnbaumer, M., and Brown, A. M.,** Molecular basis of regulation of ionic channels by G proteins, *Rec. Progr. Hormone Res.*, 45, 120. 1989.
39. **Van Dop, C., Tsubokawa, M., Bourne, H. R., and Ramachandran, J.,** Amino acid sequence of retinal transducin at the site ADP-ribosylated by cholera toxin, *J. Biol. Chem.*, 259, 696, 1984.
40. **West, R. E., Jr., Moss, J., Vaughan, M., Liu, T., and Liu, T.-Y.,** Pertussis toxin-catalyzed ADP-ribosylation of transducin. Cystein 347 is the ADP-ribose acceptor site, *J. Biol. Chem.*, 260, 14428, 1985.
41. **Scherer, N. M., Toro, M.-J., Entman, M. L., and Birnbaumer, L.,** G protein distribution in canine cardiac sarcoplasmic reticulum and sarcolemma. Comparison to rabbit skeletal membranes and brain and erythrocyte G proteins, *Arch. Biochem. Biophys.*, 259, 431, 1987.
42. **Goldsmith, P., Backlund, P. S., Jr., Rossiter, K., Carter, A., Milligan, G., Unson, C. G., and Spiegel, A.,** Purification of heterotrimeric GTP-binding proteins from brain: identification of a novel form of G_o, *Biochemistry*, 227, 7085, 1988.
43. **Mattera, R., Yatani, A., Kirsch, G. E., Graf, R., Olate, J., Codina, J., Brown, A. M., and Birnbaumer, L.,** Recombinant α_i-3 subunit of G protein activates G_k-gated K^+ channels, *J. Biol. Chem.*, 264, 465, 1989a.
44. **Cerione, R. A., Staniszewski, C., Caron, M. G., Lefkowitz, R. J., Codina, J., and Birnbaumer, L.,** A role for N_i in the hormonal stimulation of adenylate cyclase, *Nature*, 318, 293, 1985.
45. **Birnbaumer, L.,** Which G protein subunits are the active mediators in signal transduction?, *Trends Pharmacol. Sci.*, 8, 209, 1987.
46. **Kim, D., Lewis, D. L., Graziadei, L., Neer, E. J., Bar-Sagi, D., and Clapham, D. E.,** G protein βγ-subunits activate the cardiac muscarinic K^+ channel via phospholipase A_2, *Nature*, 337, 557, 1989.
47. **Kurachi, Y., Ito, H., Sugimoto, T., Shimizu, T., Miki, I., and Ui, M.,** Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K^+ channel, *Nature*, 337, 555, 1989.
48. **Tolkovsky, A. M. and Levitzki, A.,** Mode of coupling between the β-adrenergic receptor and adenylate cyclase in turkey erythrocytes, *Biochemistry*, 17, 3795, 1978.
49. **Cassel, D. and Selinger, Z.,** Mechanism of adenylate cyclase acti-

vation through the *beta*-adrenergic receptor: catecholamine-induced displacement of bound GDP by GTP, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 4155, 1978.

50. **Cassel, D., Eckstein, F., Lowe, M., and Selinger, Z.,** Determination of the turn-off reaction for the hormone-activated adenylate cyclase, *J. Biol. Chem.*, 254, 9835, 1979.
51. **Birnbaumer, L., Swartz, T. L., Abramowitz, J., Mintz, P. W., and Iyengar, R.,** Transient and steady state kinetics of the interaction of nucleotides with the adenylyl cyclase system from rat liver plasma membranes: interpretation in terms of a simple two-state model, *J. Biol. Chem.*, 255, 3542, 1980.
52. **Iyengar, R., Abramowitz, J., Riser, M., and Birnbaumer, L.,** Hormone receptor-mediated stimulation of the rat liver plasma membrane adenylyl cyclase system: nucleotide effects and analysis in terms of a two-state model for the basic receptor-affected enzyme, *J. Biol. Chem.*, 255, 3558, 1980.
53. **Iyengar, R. and Birnbaumer, L.,** Hormone receptors modulate the regulatory component of adenylyl cyclases by reducing its requirment for Mg ion and increasing its extent of activation by guanine nucleotides, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 5179, 1982.
54. **Birnbaumer, L., Hildebrandt, J. D., Codina, J., Mattera, R., Cerione, R. A., Sunyer, T., Rojas, F. J., Caron, M. G., Lefkowitz, R. J., and Iyengar, R.,** Structural basis of adenylate cyclase stimulation and inhibition by distinct guanine nucleotide regulatory proteins, in *Molecular Mechanisms of Signal Transduction*, Cohen, P. and Houslay, M. D., Eds., Elsevier/North Holland Biomedical Press, Amsterdam, 1985, 131.
55. **Asano, T., Pedersen, S. E., Scott, C. W., and Ross, E. M.,** Reconstitution of catecholamine-stimulated binding of guanosine 5'-0-(3-thiotriphosphate) to the stimulatory GTP-binding protein of adenylate cyclase, *Biochemistry*, 23, 5460, 1984.
56. **Brandt, D. R. and Ross, E. M.,** Catecholamine-stimulated GTPase cycle. Multiple sites of regulation by beta-adrenergic receptor and Mg^{2+} studied in reconstituted receptor-G_s vesicles, *J. Biol. Chem.*, 261, 1656, 1986.
57. **Watkins, P. A., Burns, D. L., Kanaho, Y., Liu, T.-Y., Hewlett, E. L., and Moss, J.,** ADP-ribosylation of transducin by pertussis toxin, *J. Biol. Chem.*, 260, 13478, 1985.
58. **Kanaho, Y., Tsai, S.-C., Adamik, R., Hewlett, E. L., Moss, J., and Vaughan, M.,** Rhodopsin-enhanced GTPase activity of the inhibitory GTP-binding protein of adenylate cyclase, *J. Biol. Chem.*, 259, 7378, 1984.
59. **Florio, V. A. and Sternweis, P. C.,** Reconstitution of resolved muscarinic cholinergic receptors with purified GTP-binding proteins, *J. Biol. Chem.*, 260, 3477, 1985.
60. **Florio, V. A. and Sternweis, P. C.,** Mechanism of muscarinic receptor action on G_o in reconstituted phospholipid vesicles, *J. Biol. Chem.*, 264, 3909, 1989.
61. **Tota, M. R., Kahler, K. R., and Schimerlik, M. I.,** Reconstitution of the purified porcine atrial muscarinic acetylcholine receptor with purified porcine atrial inhibitory guanine nucleotide binding protein, *Biochemistry*, 26, 8175, 1987.
62. **Birnbaumer, L. and Rodbell, M.,** Adenyl cyclase in fat cells. II. Hormone receptors, *J. Biol. Chem.*, 244, 3477, 1969.
63. **Rodbell, M., Birnbaumer, L., and Pohl, S. L.,** Adenyl cyclase in fat cells. III. Stimulation by secretin and the effects of trypsin on the receptors for lipolytic hormones, *J. Biol. Chem.*, 245, 718, 1970.
64. **Citri, Y. and Schramm, M.,** Resolution, reconstitution and kinetics of the primary action of hormone receptor, *Nature*, 287, 297, 1975.
65. **Ross, E. M., Howlett, A. C., Ferguson, K. M., and Gilman, A. G.,** Reconstitution of hormone-sensitive adenylate cyclase activity with resolved components of the enzyme, *J. Biol. Chem.*, 253, 6401, 1978.
66. **Kaslow, H. R., Farfel, Z., Johnson, G. L., and Bourne, H. R.,** Adenylate cyclase assembled *in vitro*: cholera toxin substrates determine different patterns of regulation by isoproterenol and guanosine 5'-triphosphate, *Mol. Pharmacol.*, 15, 472, 1979.
67. **Ashkenazi, A., Winslow, J. W., Peralta, E. G., Peterson, G. L., Schimerlik, M. I., Capon, D. J., and Ramachandran, J.,** An M2 muscarinic receptor subtype coupled to both adenylyl cyclase and phosphoinositide turnover, *Science*, 238, 672, 1987.
68. **Murad, F., Chi, Y.-M., Rall, T. W., and Sutherland, E. W.,** Adenyl cyclase. III. The effect of catecholamines and choline esters on the formation of adenosine 3',5'-phosphate by preparations from cardiac muscle and liver, *J. Biol. Chem.*, 237, 1233, 1962.
69. **Marinetti, G. C., Ray, T. K., and Tomasi, V.,** Glucagon and epinephrine stimulation of adenyl cyclase in isolated rat liver plasma membranes, *Biochem. Biophys. Res. Commun.*, 36, 185, 1969.
70. **Benovic, J. L., Shorr, R. G. L., Caron, M. G., and Lefkowitz, R. J.,** The mammalian $beta_2$-adrenergic receptor: purification and properties, *Biochemistry*, 23, 4510, 1984.
71. **Makman, M. H.,** Properties of adenylate cyclase of lymphoid cells, *Proc. Natl. Acad. Sci. U.S.A.*, 68, 885, 1971.
72. **Amitai, G., Brown, R. D., and Taylor, P.,** The relationship between $alpha_1$-adrenergic receptor occupation and the mobilization of intracellular calcium *J. Biol. Chem.*, 259, 12519, 1984.
73. **Tolbert, M. E. M., White, A. C., Aspry, K., Cutts, J., and Fain, J. N.,** Stimulation by vasopressin and alpha-catecholamines of phosphatidylinositol formation in isolated rat liver parenchymal cells, *J. Biol. Chem.*, 255, 1938, 1980.
74. **Lynch, C. J., Charest, R., Blackmore, P. F., and Exton, J. H.,** Studies on the hepatic $alpha_1$-adrenergic receptor. Modulation of guanine nucleotide effects by calcium, temperature, and age, *J. Biol. Chem.*, 260, 1593, 1985.
75. **Burch, R. M., Luini, A., and Axelrod, J.,** Phospholipase A_2 and phospholipase C are activated by distinct GTP-binding proteins in response to $alpha_1$-adrenergic stimulation in FRTL-5 cells, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 7201, 1986.
76. **Bocckino, S. B., Blackmore, P. F., Wilson, P. B., and Exton, J. H.,** Phosphatidate accumulation in hormone-treated hepatocytes via a phospholipase D mechanism, *J. Biol. Chem.*, 262, 15309, 1987.
77. **Jakobs, K. H., Saur, W., and Schultz, G.,** Reduction of adenylate cyclase activity of human platelets by the *alpha*-adrenergic component of epinephrine, *J. Cyclic Nucl. Res.*, 2, 381, 1976.
78. **Tsai, B. S. and Lefkowitz, R. J.,** Agonist-specific effects of guanine nucleotides on alpha-adrenergic receptors in human platelets, *Mol. Pharmacol.*, 16, 61, 1979.
79. **Aktories, K., Schultz, G., and Jakobs, K. H.,** Inhibition of hamster fat cell adenylate cyclase by prostaglandin E^1 and epinephrine: requirement for GTP and sodium ions, *FEBS Lett.*, 107, 100, 1979.
80. **Kather, H. and Simon, B.,** Stimulatory and inhibitory effects of catecholamines and prostaglandin E_2 on human fat cell adenylate cyclase, *Adv. Cyclic Nucl. Res.*, 14, 555, 1981.
81. **Jard, S., Cantau, B., and Jakobs, K. H.,** Angiotensin II and $alpha_2$-adrenergic agonists inhibit rat liver adenylate cyclase, *J. Biol. Chem.*, 256, 2603, 1981.
82. **Dunlap, K. and Fischbach, G. D.,** Neurotransmitters decrease the calcium component of sensory neurone action potentials, *Nature*, 276, 8837, 1978.
83. **Kebabian, J. W., Petzold, G. L., and Greengard, P.,** Dopamine-sensitive adenylate cyclase in gaudate nucleus of rat brain, and its similarity to the dopamine receptor, *Proc. Natl. Acad. Sci. U.S.A.*, 69, 2145, 1971.
84. **Attie, M. F., Brown, E. M., Gardner, D. G., Spiegel, A. M., and Aurbach, G. D.,** Characterization of the dopamine-responsive adenylate cyclase of bovine parathyroid cells and its relationship to parathyroid hormone secretion, *Endocrinology*, 107, 1176, 1980.
85. **DeCamilli, P., Macconi, D., and Spada, A.,** Dopamine inhibits

adenylate cyclase in human prolactin-secreting adenomas, *Nature*, 278, 252, 1979.

86. **Cote, T. E., Grewe, C. W., Tsuruta, K., Stoof, J. C., Eskay, R. L., and Kebabian, J. W.,** D-2 dopamine receptor-mediated inhibition of adenylate cyclase activity in the intermediate lobe of the rat pituitary gland requires guanosine 5'-triphosphate, *Endocrinology*, 110, 812, 1982.
87. **Rubin, R. P., Godfrey, P. P., Chapman, D. A., and Putney, J. W., Jr.,** Secretagogue-induced formation of inositol phosphates in rat exocrine pancreas. Implications for a messenger role for inositol triphosphate, *Biochem. J.*, 219, 655, 1984.
88. **Berridge, M. J.,** Rapid accumulation of inositol trisphosphate reveals that agonists hydrolyze polyphosphoinositides instead of phosphatidylinositol, *Biochem. J.*, 212, 849, 1983.
89. **Adams, P. R., Brown, D. A., and Constanti, A.,** Pharmacological inhibition of the M-current, *J. Physiol. (London)*, 332, 223, 1982.
90. **Mattera, R., Pitts, B. J. R., Entman, M. S., and Birnbaumer, L.,** Guanine nucleotide regulation of a mammalian myocardial receptor system. Evidence for homo- and heterotropic cooperativity in ligand binding analyzed by computer assisted curve fitting, *J. Biol. Chem.*, 260, 7410, 1985.
91. **Egan, T. M. and North, R. A.,** Acetylcholine hyperpolarizes central neurones by acting on an M_2 muscarinic receptor, *Nature*, 319, 405, 1986.
92. **Andrade, R., Malenka, R. C., and Nicoll, R. A.,** A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus, *Science*, 234, 1261, 1986.
93. **Wojcik, W. J. and Neff, N. H.,** *gamma*-Aminobutyric acid B receptors are negatively coupled to adenylate cyclase in brain, and in the cerebellum these receptors may be associated with granule cells, *Mol. Pharmacol.*, 25, 24, 1984.
94. **Cooper, D. M. F., Londos, C., and Rodbell, M.,** Adenosine receptor-mediated inhibition of rat cerebral cortical adenylate cyclase by a GTP-dependent process, *Mol. Pharmacol.*, 18, 598, 1980.
95. **Londos, C., Cooper, D. M. F., Schlegel, W., and Rodbell, M.,** Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process: basis for actions of adenosine and methylxanthines on cyclic AMP production and lipolysis, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 5362, 1978.
96. **Kurachi, Y., Nakajima, T., and Sugimoto, T.,** On the mechanism of activation of muscarinic K^+ channels by adenosine in isolated atrial cells: involvement of GTP-binding proteins, *Pflügers Arch.*, 407, 264, 1986.
97. **Butcher, R. W., Baird, C. E., and Sutherland, E. W.,** Effects of lipolytic and antilipolytic substances on adenosine 3',5'-monophosphate levels in isolated fat cells, *J. Biol. Chem.*, 243, 1705, 1968.
98. **Birnbaumer, L., Nakahara, T., and Yang, P.-Ch.,** Studies on receptor-mediated activation of adenylyl cyclases. II. Nucleotide and nucleoside regulations of the activites of the beef renal medullary adenylyl cyclase and their stimulation by neurohypophyseal hormones, *J. Biol. Chem.*, 249, 7857, 1984.
99. **Blume, A. J. and Foster, C. J.,** Mouse neuroblastoma adenylate cyclase. Adenosine and adenosine analogues as potent effectors of adenylate cyclase activity, *J. Biol. Chem.*, 250, 5003, 1975.
100. **Boyer, J. L., Downes, C. P., and Harden, T. K.,** Kinetics of activation of phospholipase C by P_{2Y}-purinergic receptor agonists and guanine nucleotides, *J. Biol. Chem.*, 264, 884, 1988.
101. **Irving, H. R. and Exton, J. H.,** Phosphatidylcholine breakdown in rat liver plasma membranes. Roles of guanine nucleotides and P_2-purinergic agonists, *J. Biol. Chem.*, 262, 3440, 1987.
102. **Weiss, S., Sebben, M., Kemp, D. E., and Bockaert, J.,** Serotonin $5\text{-}HT_1$ receptors mediate inhibition of cyclic AMP production in neurons, *Eur. J. Pharmacol.*, 120, 227, 1986.
103. **Doyle, V. M., Creba, J. A., Rüegg, U. T., and Hoyer, D.,** Serotonin increases the production of inositol phosphates and mobilises calcium via the $5\text{-}HT_2$ receptor in A_7r_5 smooth muscle cells, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 333, 98, 1986.
104. **Garber, A. J.,** The impact of streptozotocin-induced diabetes mellitus on cyclic nucleotide regulation of skeletal muscle amino acid metabolism in the rat, *J. Clin. Invest.*, 65, 478, 1980.
105. **Enjalbert, A., Bourgoin, S., Hamon, M., Adrien, J., and Bockaert, J.,** Postsynaptic serotonin-sensitive adenylate cyclase in the central nervous system. I. Development and distribution of serotonin and dopamine-sensitive adenylate cyclases in rat and guinea pig brain, *Mol. Pharmacol.*, 14, 2, 1978.
106. **Daum, P. R., Downes, C. P., and Young, J. M.,** Histamine stimulated inositol 1-phosphate accumulation in lithium-treated slices from regions of a pig brain, *J. Neurochem.*, 43, 25, 1984.
107. **Noble, E. P., Bommer, M., Sincini, E., Costa, T., and Herz, A.,** Histaminergic activation stimulates inositol-1-phosphate accumulation in chromaffin cells, *Biochem. Biophys. Res. Commun.*, 135, 566, 1986.
108. **Klein, I. and Levey, G. S.,** Activation of myocardial adenyl cyclase by histamine in guinea pig, cat and human heart, *J. Clin. Invest.*, 50, 1012, 1971.
109. **Hegstrand, L. R., Kanof, P. D., and Greengard, P.,** Histamine-sensitive adenylate cyclase in mammalian brain, *Nature*, 260, 163, 1976.
110. **McNeill, J. H. and Verma, S. C.,** Stimulation of rat gastric adenylate cyclase by histamine and histamine analogues and blockade by burimamide, *Br. J. Pharmacol.*, 52, 104, 1974.
111. **Taunton, D. O., Roth, J., and Pastan, I.,** ACTH stimulation of adenyl cyclase in adrenal homogenates, *Biochem. Biophys. Res. Commun.*, 29, 1, 1967.
112. **Kojima, I. and Ogata, E.,** Direct demonstration of adrenocorticotropin-induced changes in cytoplasmic free calcium with aequorin in adrenal glomerulosa cell, *J. Biol. Chem.*, 261, 9832, 1986.
113. **Schrey, M. P. and Rubin, R. P.,** Characterization of a calcium-mediated activation of arachidonic acid turnover in adrenal phospholipids by corticotropin, *J. Biol. Chem.*, 254, 11234, 1979.
114. **Sharma, S. K., Nirenberg, M., and Klee, W. A.,** Morphine receptors as regulators of adenylate cyclase activity, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 590, 1975.
115. **Blume, A. J.,** Interaction of ligands with the opiate receptors of brain membranes: regulation by ions and nucleotides, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 1713, 1978.
116. **Abramowitz, J. and Campbell, A. R.,** Enkephalin-mediated inhibition of forskolin-stimulated rabbit luteal adenylyl cyclase activity, *Biochem. Biophys. Res. Commun.*, 116, 574, 1983.
117. **Mudge, A. W., Leeman, S. E., and Fischbach, G. D.,** Enkephalin inhibits release of substance P from sensory neurons in culture and decreases action potential duration, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 526, 1979.
118. **Attali, B., Saya, D., Nah, S.-Y., and Vogel, Z.,** *Kappa* opiate agonists inhibit Ca^{2+} influx in rat spinal cord-dorsal root ganglion cocultures. Involvement of a GTP-binding protein, *J. Biol. Chem.*, 264, 347, 1989.
119. **Williams, J. T., Egan, T. M., and North, R. A.,** Enkephalin opens potassium channels on mammalian central neurons, *Nature*, 299, 74, 1982.
120. **Kolena, J. and Channing, C. P.,** Stimulatory effects of LH, FSH and prostaglandins upon cyclic 3',5'-AMP in porcine granulosa cells, *Endocrinology*, 90, 1543, 1972.
121. **Marsh, J. M., Butcher, R. W., Savard, K., and Sutherland, E. W.,** The stimulatory effect of leuteinizing hormone on adenosine 3',5'-monophosphate accumulation in corpus luteum, *J. Biol. Chem.*, 241, 5436, 1966.
122. **Murad, F., Strauch, B. S., and Vaughan, M.,** The effect of gonadotropins on testicular adenyl cyclase, *Biochim. Biophys. Acta*, 177,

591, 1969.

123. **Davis, J. S., Weakland, L. L., Farese, R. V., and West, L. A.,** Luteinizing hormone increases inositol trisphophate and cytosolic free Ca^{2+} in isolated bovine luteal cells, *J. Biol. Chem.*, 262, 8515, 1987.
124. **Klainer, L. M., Chi, Y.-M., Friedberg, S. L., Rall, T. W., and Sutherland, E. E.,** Adenyl cyclase. IV. The effects of neurohormones on the formation of adenosine 3′,5′-phosphate by preparations from brain and other tissues, *J. Biol. Chem.*, 237, 1239, 1962.
125. **Abe, K., Butcher, R. W., Nicholson, W. E., Baird, C. E., Liddle, R. A., and Liddle, G. W.,** Adenosine 3′,5′-monophosphate (cyclic AMP) as the mediator of the actions of melanocyte stimulating hormone (MSH) and norepinephrine on the frog skin, *Endocrinology*, 84, 362, 1969.
126. **Labrie, F., Gagne, B., and Lefevre, G.,** Corticotropin-releasing factor stimulates adenylate cyclase activity in the anterior pituitary, *Life Sci.*, 31, 1117, 1982.
127. **Bilezikjian, L. M. and Vale, W. W.,** Stimulation of adenosine 3′,5′-monophosphate production by growth hormone-releasing factor and its inhibition by somatostatin in anterior pituitary cells *in vitro*, *Endocrinology*, 113, 1726, 1983.
128. **Schettini, G., Cronin, M. J., Hewlett, E. L., Thorner, M. O., and MacLeod, R. M.,** Human pancreatic tumor growth hormone-releasing factor stimulates anterior pituitary adenylate cyclase activity, adenosine 3′,5′-monophosphate accumulation and growth hormone release in a calmodulin-dependent manner, *Endocrinology*, 115, 1308, 1984.
129. **Naor, Z. and Catt, K. J.,** Mechanism of action of gonadotropin-releasing hormone. Involvement of phospholipid turnover in luteinizing hormone release, *J. Biol. Chem.*, 256, 2226, 1981.
130. **Clark, M. R., Thibier, C., Marsh, J. M., and LeMaire, W. J.,** Stimulation of prostaglandin accumulation by luteinizing hormone-releasing hormone (LHRH) and LHRH analogs in rat granulosa cells *in vitro*, *Endocrinology*, 107, 17, 1980.
131. **Molocho, J., Zakut, H., and Naor, Z.** Gonadotropin-releasing hormone stimulates phosphatidylinositol labeling and prostaglandin E production in leydig cells, *Endocrinology*, 114, 1048, 1984.
132. **Snyder, G. D. and Bleasdale, J. E.,** Effect of LHRH on incorporation of [^{32}P]-orthophosphate into phosphatidylinositol by dispersed anterior pituitary cells, *Mol. Cell. Endocrinol.*, 28, 55, 1982.
133. **Naor, Z. and Yavin, E.,** Gonadotropin-releasing hormone stimulates phospholipid labeling in cultured granulosa cells, *Endocrinology*, 111, 1615, 1982.
134. **Leung, P. C. K., Raymond, V., and Labrie, F.,** Stimulation of phosphatidic acid and phosphatidylinositol labeling in luteal cells by luteinizing hormone releasing hormone, *Endocrinology*, 112, 1138, 1983.
135. **Martin, T. F. J.,** Thyrotropin-releasing hormone rapidly activates the phosphodiester hydrolysis of polyphosphoinositides in GH_3 pituitary cells. Evidence for the role of aa polyphosphoinositide-specific phospholipase C in hormone action, *J. Biol. Chem.*, 258, 14816, 1983.
136. **Rebecchi, M. J. and Gershengorn, M. C.,** Thyroliberin stimulates rapid hydrolysis of phosphatidylinositol 4,5-bisphosphate by a phosphodiesterase in rat mammotropic pituitary cells. Evidence for an early Ca^{2+}-independent action, *Biochem. J.*, 216, 287, 1983.
137. **Kolesnick, R. N., Musacchio, L., Thaw, C., and Gershengorn, M. C.,** Thyrotropin (TSH)-releasing hormone decreases phosphatidylinositol and increases unesterified arachidonic acid in thyrotropic cells: possible early events in stimulation of TSH secretion, *Endocrinology*, 114, 671, 1984.
138. **Toro, J. and Birnbaumer, L.,** Inhibitory regulation of GH_4 cell adenylyl cyclase by TRH and differentiation from that of somatostatin using cholera and pertussis toxins. Possible evidence of an action of another nucleotide regulatory component, *Endocrinology*, 118 (Suppl. 1), Abstr. 415, 1986.
139. **Koch, B. D. and Schonbrunn, A.,** The somatostatin receptor is directly coupled to adenylate cyclase in GH_4C_1 pituitary cell membranes, *Endocrinology*, 114, 1784, 1984.
140. **Cronin, M. J., Rogol, A. D., Myers, G. A., and Hewlett, E. L.,** Pertussis toxin blocks the somatostatin-induced inhibition of growth hormone release and adenosine 3′,5′-monophosphate accumulation, *Endocrinology*, 113, 209, 1983.
141. **Heisler, S., Reisine, T. D., Hook, V. Y. H., and Axelrod, J.,** Somatostatin inhibits multireceptor stimulation of cyclic AMP formation and corticotropin secretion in mouse pituitary tumor cells, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 6502, 1982.
142. **Jakobs, K. H., Aktories, K., and Schultz, G.,** A nucleotide regulatory site for somatostatin inhibition of adenylate cyclase in S49 lymphoma cells, *Nature*, 303, 177, 1983.
143. **Vinicor, F., Higdon, G., and Clark, C. M., Jr.,** Effects of somatostatin on the hepatic adenylate cyclase system in the rat, *Endocrinology*, 101, 1071, 1977.
144. **Bone, E. A., Fretten, P., Palmer, S., Kirk, C. J., and Michell, R. H.,** Rapid accumulation of inositol phosphates in isolated rat superior cervical sympathetic ganglia exposed to V_1-vasopressin and muscarinic cholinergic stimuli, *Biochem. J.*, 221, 803, 1984.
145. **Pfeilschifter, J., Kurtz, A., and Bauer, C.,** Activation of phospholipase C and prostaglandin synthesis by (arginine) vasopressin in cultures of rat renal mesangial cells, *Biochem. J.*, 223, 855, 1984.
146. **Morgan, N. G., Shipp, C. C., and Exton, J. H.,** Studies on the mechanism of inhibition of hepatic cAMP accumulation by vasopressin, *FEBS Lett.*, 163, 277, 1983.
147. **Guillon, G., Gaillard, R. C., Kehrer, P., Shoenenberg, P., Muller, A. F., and Jard, S.,** Vasopressin and angiotensin induce inositol lipid breakdown in rat adenohypophysial cells in primary culture, *Reg. Peptides*, 18, 119, 1987.
148. **Brown, E., Clarke, D. L., Roux, V., and Sherman, G. H.,** The stimulation of adenosine 3′,5′-monophosphate production by antidiuretic factors, *J. Biol. Chem.*, 238, 852, 1963.
149. **Marc, S., Leiber, D., and Harbon, S.,** Carbachol and oxytocin stimulate the generation of inositol phosphates in the guinea pig myometrium, *FEBS Lett.*, 201, 9, 1986.
150. **Lee, C. Y.,** Modulation of ovarian adenylate cyclase response to gonadotropins following luteinization of the rat ovary, *Endocrinology*, 101, 876, 1977.
151. **Tsuruhara, T., Dufau, M. L., Cigorraga, S., and Catt, K. J.,** Hormonal regulation of testicular luteinizing hormone receptors. Effects on cyclic AMP and testosterone responses in isolated Leydig cells, *J. Biol. Chem.*, 252, 9002, 1977.
152. **Makman, M. H. and Sutherland, E. W.,** Use of liver adenyl cyclase for assay of glucagon in human gastrointestinal tract and pancreas, *Endocrinology*, 75, 127, 1964.
153. **Levey, G. S. and Epstein, S. E.,** Activation of adenyl cyclase by glucagon in cat and human heart, *Circulation Res.*, 24, 151, 1969.
154. **Murad, F. and Vaughan, M.,** Effect of glucagon on rat heart adenyl cyclase, *Biochem. Pharmacol.*, 18, 1053, 1969.
155. **Goldfine, I., Roth, J., and Birnbaumer, L.,** Glucagon receptor in beta cells: binding of ^{125}I-glucagon and activation of adenylate cyclase, *J. Biol. Chem.*, 247, 1211, 1972.
156. **Lotersztajn, S., Epand, R. M., Mallat, A., and Pecker, F.,** Inhibition by glucagon of the calcium pump in liver plasma membranes, *J. Biol. Chem.*, 259, 8195, 1984.
157. **Wakelam, M. J. O., Murphy, G. J., Hruby, V. J., and Houslay, M. D.,** Activation of two signal-transduction systems in hepatocytes by glucagon, *Nature*, 323, 68, 1986.
158. **Long, B. W. and Gardner, J. D.,** Effects of cholecystokinin on adenylate cyclase activity in dispersed pancreatic acinar cells, *Gastroenterology*, 73, 1008, 1977.
159. **Shelby, H. T., Gross, L. P., Lichty, P., and Gardner, J. D.,** Acf cholecystokinin and cholinergic agents on membrane-bound calcium

in dispersed pancreatic acinar cells, *J. Clin. Invest.*, 58, 1482, 1976.
160. **Robberecht, P., Conlon, T. P., and Gardner, J. D.,** Interaction of porcine vasoactive intestinal peptide with dispersed pancreatic acinar cells from the guinea pig. Structural requirements for effects of vasoactive intestinal peptide and secretin on cellular adenosine 3′:5′-monophosphate, *J. Biol. Chem.*, 251, 4635, 1976.
161. **Deschodt-Lanckman, M., Robberecht, P., and Christophe, J.,** Characterization of VIP-sensitive adenylate cyclase in guinea pig brain, *FEBS Lett.*, 83, 76, 1977.
162. **Waldman, D. B., Gardner, J. D., Zfass, A. M., and Makhlous, G. M.,** Effects of vasoactive intestinal peptide, secretin, and related peptides on rat colonic transport and adenylate cyclase activity, *Gastroenterology*, 73, 518, 1977.
163. **Audigier, S., Barberis, C., and Jard, S.,** Vasoactive intestinal polypeptide increases inositol phospholipid breakdown in the rat superior cervical ganglion, *Brain Res.*, 376, 363, 1986.
164. **Chase, L. R. and Aurbach, G. D.,** Renal adenyl cyclase: anatomically separate sites for vasopressin and parathyroid hormone, *Science*, 159, 545, 1968.
165. **Hruska, K. A., Moskowitz, D., Esbrit, P., Civitelli, R., Westbrook, S., and Huskey, M.,** Stimulation of inositol trisphosphate and diacylglycerol production in renal tubular cells by parathyroid hormone, *J. Clin. Invest.*, 79, 230, 1987.
166. **Farese, R. V., Larson, R. E., Sabir, M. A., and Gomez-Sanchez, C.,** Effects of angiotensin-II and potassium on phospholipid metabolism in the adrenal zona glomerulosa, *J. Biol. Chem.*, 256, 11093, 1981.
167. **Alexander, R. W., Brock, T. A., Gimbrone, M. A., Jr., and Rittenhouse, S. E.,** Angiotensin increases inositol trisphosphate and calcium in vascular smooth muscle, *Hypertension*, 7, 447, 1985.
168. **Marie, J. and Jard, S.,** Angiotensin II inhibits adenylate cyclase from adrenal cortex glomerulosa zone, *FEBS Lett.*, 159, 97, 1983.
169. **Khanum, Z. and Dufau, M. L.,** Angiotensin II receptors and inhibitory actions in Leydig cells, *J. Biol. Chem.*, 263, 5070, 1988.
170. **Woodcock, E. A. and Johnston, C. I.,** Inhibition of adenylate cyclase by angiotensin II in rat renal cortex, *Endocrinology*, 111, 1687, 1982.
171. **Benabet, J. E., Spry, L. A., and Morrison, A. R.,** Effects of angiotensin II on phosphatidylinositol and polyphosphoinositide turnover in rat kidney. Mechanism of prostaglandin release, *J. Biol. Chem.*, 257, 7430, 1982.
172. **Murayama, T. and Ui, M.,** Receptor-mediated inhibition of adenylate cyclase and stimulation of arachidonic acid release in 3T3 fibroblasts. Selective susceptibility to islet-activating protein, pertussis toxin, *J. Biol. Chem.*, 260, 7226, 1985.
173. **Murad, F., Brewer, H. B., Jr., and Vaughan, M.,** Effect of thyrocalcitronin on adenosine 3′:5′-cyclic phosphate formation by rat kidney and bone, *Proc. Natl. Acad. Sci. U.S.A.*, 65, 446, 1970.
174. **Laufer, R. and Changeux, J.-P.,** Calcitonin gene-related peptide elevates cyclic AMP levels in chick skeletal muscle: possible neurotrophic role for a coexisting neuronal messenger, *EMBO J.*, 6, 901, 1987.
175. **Laufer, R. and Changeaux, J.-P.,** Calcitonin gene-related peptide and cyclic AMP stimulate phosphoinositide turnover in skeletal muscle. Interaction between two second messenger systems, *J. Biol. Chem.*, 264, 2683, 1989.
176. **Volpi, M., Yassin, R., Naccache, P. H., and Sha'afi, R. I.,** Chemotactic factor causes rapid decreases in phosphatidylinositol, 4,5-bisphosphate and phosphatidylinositol 4-monophosphate in rabbit neutrophils, *Biochem. Biophys. Res. Commun.*, 112, 957, 1983.
177. **Rubin, R. P., Sink, L. E., and Freer, R. J.,** On the relationship between formyl-methionyl-leucyl-phenylalanine stimulation of arachidonyl phosphatidylinositol turnover and lysosomal enzyme secretion by rabbit neutrophils, *Mol. Pharmacol.*, 19, 31, 1981.
178. **Billah, M. M. and Lapetina, E. G.,** Rapid decrease of phosphatidylinositol 4,5-bisphosphate in thrombin-stimulated platelets, *J. Biol. Chem.*, 257, 12705, 1982.
179. **Bell, R. L., Kennerly, D. A., Stanford, N., and Majerus, P. W.,** Di-glyceride lipase: a pathway for arachidonate release from human platelets, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 3238, 1979.
180. **Wakelam, M. J. O., Davies, S. A., Houslay, M. D., McKay, I., Marshall, C. J., and Hall, A.,** Normal $p21^{N\text{-}ras}$ couples bombesin and other growth factor receptors to inositol phosphate production, *Nature*, 323, 173, 1986.
181. **Berridge, M. J., Heslop, J. P., Irvine, R. F., and Brown, K. D.,** Inositol trisphosphate formation and calcium mobilization in Swiss 3T3 cells in response to platelet-derived growth factor, *Biochem. J.*, 222, 195, 1984.
182. **Beaven, M. A., Moore, J. P., Smith, G. A., Hesketh, T. R., and Metcalfe, J. C.,** The calcium signal and phosphatidylinositol breakdown in 2H3 cells, *J. Biol. Chem.*, 259, 7137, 1984.
183. **Crews, F. T., Morita, Y., Hirata, F., Axelrod, J., and Siraganian, R. P.,** Phospholipid methylation affects immunoglobulin E-mediated histamine and arachidonic acid release in rat leukemic basophils, *Biochem. Biophys. Res. Commun.*, 93, 42, 1980.
184. **Higashida, H., Streaty, R. A., Klee, W., and Nirenberg, M.,** Bradykinin-activated transmembrane signals are coupled via N_o or N_i to production of inositol 1,4,5-trisphosphate, a second messenger in NG108-15 neuroblastoma-glioma hybrid cells, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 942, 1986.
185. **Lambert, T. L., Kent, R. S., and Whorton, A. R.,** Bradykinin stimulation of inositol polyphosphate production in porcine aortic endothelial cells, *J. Biol. Chem.*, 261, 15288, 1986.
186. **Shayman, J. A. and Morrison, A. R.,** Bradykinin-induced changes in phosphatidyl inositol turnover in cultured rabbit papillary collecting tubule cells, *J. Clin. Invest.*, 76, 978, 1985.
187. **Hong, S. L. and Deykin, D.** Activation of phospholipase A_2 and C in pig aortic endothelial cells synthesizing prostacyclin, *J. Biol. Chem.*, 257, 7151, 1982.
188. **Schwartzman, M., Liberman, E., and Raz, A.,** Bradykinin and angiotensin II activation of arachidonic acid deacylation and prostaglandin F_2 formation in rabbit kidney, *J. Biol. Chem.*, 256, 2329, 1981.
189. **Higashida, H. and Brown, D. A.,** Two polyphosphatidylinositide metabolites control two K^+ currents in neuronal cell, *Nature*, 323, 333, 1986.
190. **Watson, S. P. and Downes, C. P.,** Substance P induced hydrolysis of inositol phospholipids in guinea-pig ileum and rat hypothalamus, *Eur. J. Pharmacol.*, 93, 245, 1983.
191. **Hanley, M. R., Lee, C. M., Jones, L. M., and Michell, R. H.,** Similar effects of substance P and related peptides on salivation and on phosphatidylinositol turnover in rat salivary glands, *Mol. Pharmacol.*, 18, 78, 1980.
192. **Yousufzai, S. Y. K., Akhtar, R. A., and Abdel-Latif, A. A.,** Effects of substance P on inositol trisphosphate accumulation, on contractile responses, and on arachidonic acid release and prostaglandin biosynthesis in rabbit iris sphincter muscle, *Exp. Eye Res.*, 43, 215, 1986.
193. **Kassis, S., Olasmaa, M., Terenius, L., and Fishman, P. H.,** Neuropeptide Y inhibits cardiac adenylate cyclase through a pertussis toxin-sensitive G protein, *J. Biol. Chem.*, 262, 3429, 1987.
194. **Inui, A., Okita, M., Inoue, T., Sakatani, N., Oya, M., Morioka, H., Shii, K., Yokono, K., Mizuno, N., and Baba, S.,** Characterization of peptide YY receptors in the brain, *Endocrinology*, 124, 402, 1989.
195. **Imamura, K., Sherman, M. L., Spriggs, D., and Kufe, D.,** Effect of tumor necrosis factor on GTP binding an GTPase activity in HL-60 and L929 cells, *J. Biol. Chem.*, 263, 1, 1988.
196. **Imamura, K. and Kufe, D.,** CSF-1 induced Na^+ influx into human monocytes involves activation of a pertussis toxin sensitive GTP-binding protein, *J. Biol. Chem.*, 263, 14093, 1988.

197. **Rosoff, P. M., Savage, N., and Dinarello, C. A.,** Interleukin-1 stimulates diacylglycerol production in T lymphocytes by a novel mechanism, *Cell*, 54, 73, 1988.
198. **Goedert, M., Pinnock, R. D., Downes, C. P., Mantyh, P. W., and Emson, P. C.,** Neurotensin stimulates inositol phospholipid hydrolysis in rat brain slices, *Brain Res.*, 323, 193, 1984.
199. **Bozou, J.-C., Amar, S., Vincent, J.-P., and Kitabgi, P.,** Neurotensin-mediated inhibition of cyclic AMP formation in neuroblastoma N1E115 cells: involvement of the inhibitory GTP-binding component of adenylate cyclase, *Mol. Pharmacol.*, 29, 489, 1986.
200. **Anand-Srivastava, M. B., Srivastata, A. K., and Cantin, M.,** Pertussis toxin attenuates atrial natriuretic factor-mediated inhibition of adenylate cyclase. Involvement of inhibitory guanine nucleotide regulatory protein, *J. Biol. Chem.*, 262, 4931, 1987.
201. **Moolenaar, W. H., Kruijer, W., Tilly, B. C., Verlaan, I., Bierman, A. J., and de Laat, S. W.,** Growth factor-like action of phosphatidic acid, *Nature*, 323, 171, 1986.
202. **Haslam, R. J. and Vanderwel, M.,** Inhibition of platelet adenylate cyclase by 1-O-alkyl-2-O-acetyl-*sn*-glyceryl-3-phosphorylcholine (platelet-activating factor), *J. Biol. Chem.*, 257, 6879, 1982.
203. **Shukla, S. D., Buxton, D. B., Olson, M. S., and Hanahan, D. J.,** Acetyl-glyceryl ether phosphorylcholine: a potent activator of hepatic phosphoinositide metabolism and glycogenolysis, *J. Biol. Chem.*, 258, 10212, 1983.
204. **Amiranoff, B., Lorinet, A. M., Lagny-Pourmir, I., and Laburthe, M.,** Mechanism of galanin-inhibited insulin release. Occurrence of a pertussis-toxin-sensitive inhibition of adenylate cyclase, *Eur. J. Biochem.*, 177, 147, 1988.
205. **Ueda, H., Yoshihara, Y., Misawa, H., Fukushima, N., Katada, T., Ui, M., Takagi, H., and Satoh, M.,** The kyotorphin (tyrosine-arginine) receptor and a selective reconstitution with purified G_i, measured with GTPase and phospholipase C assays, *J. Biol. Chem.*, 264, 3732, 1989.
206. **Kather, H. and Simon, B.,** Biphasic effects of prostaglandin E_2 on the human fat cell adenylate cyclase, *J. Clin. Invest.*, 64, 609, 1979.
207. **Marumo, F. and Edelman, I. S.,** Effects of Ca^{++} and prostaglandin E_1 on vasopressin activation of renal adenyl cyclase, *J. Clin. Invest.*, 50, 1613, 1971.
208. **Davis, J. S., Weakland, L. L., Weiland, D. A., Farese, R. B., and West, L. A.,** Prostaglandin $F_{2\alpha}$ stimulates phosphatidylinositol 4,5-bisphosphate hydrolysis and mobilizes intracellular Ca^{2+} in bovine luteal cells, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 3728, 1987.
209. **Marsh, M. M.,** The stimulating effect of prostaglandin E_2 on adenyl cyclase in the bovine corpus luteum, *FEBS Lett.*, 7, 283, 1980.
210. **Abramowitz, J. and Birnbaumer, L.,** Prostacyclin activation of adenylyl cyclase in rabbit corpus luteum membranes: comparison with 6-keto prostaglandin F_1*alpha* and prostaglandin E_1, *Biol. Reprod.*, 21, 609, 1979.
211. **Birnbaumer, L. and Yang, P.-Ch.,** Studies on receptor-mediated activation of adenylyl cyclases. III. Regulation by purine nucleotides of the activation of adenylyl cyclases from target organs for prostaglandins, luteinizing hormone, neurohypophyseal hormones and catecholamines. Tissue and hormone-dependent variations, *J. Biol. Chem.*, 249, 7867, 1974.
212. **Cramer, E. B., Pologe, L., Pawlowski, N. A., Cohn, Z. A., and Scott, W. A.,** Leukotriene C promotes prostacyclin synthesis by human endothelial cells, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 4109, 1983.
213. **Clark, M. A., Conway, T. M., Bennett, C. F., Crooke, S. T., and Stadel, J. M.,** Islet-activating protein inhibits leukotriene D_4 and leukotriene C_4 but not bradykinin- or calcium ionophore-induced prostacyclin synthesis in bovine endothelial cells, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 7320, 1986.
214. **Sarau, H. M., Mong, S., Foley, J. J., Wu, H.-L., and Crooke, S. T.,** Identification and characterization of leukotriene D_4 receptors and signal transduction processes in rat basophilic leukemia cells, *J. Biol. Chem.*, 262, 4034, 1987.
215. **Wheeler, G. L. and Bitensky, M. W.,** A light-activated GTPase in vertebrate photoreceptors: regulation of light-activated cyclic GMP phosphodiesterase, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 4238, 1977.
216. **Pace, U., Hanski, E., Salomon, Y., and Lancet, D.,** Odorant-sensitive adenylate cyclase may mediate olfactory reception, *Nature*, 316, 255, 1985.
216a. **Berger, S. J., DeVries, G. W., Carter, J. G., Schulz, D. W., Passonneau, P. N., Lowry, O. H., and Ferrendelli, J. A.,** The distribution of the components of the cyclic GMP cycle in retina, *J. Biol. Chem.*, 255, 3128, 1980.
217. **Huque, T. and Bruch, R. C.,** Odorant- and guanine nucleotide-stimulated phosphoinositide turnover in olfactory cilia, *Biochem. Biophys. Res. Commun.*, 137, 36, 1986.
218. **Birnbaumer, L., Abramowitz, J., and Brown, A. M.,** Signal transduction by G proteins, *Biochem. Biophys. Acta Rev. Biomembr.*, in press, 1989.
219. **Suki, W., Abramowitz, J., Mattera, R., Codina, J., and Birnbaumer, L.,** The human genome encodes at least three non-allelic G proteins with alpha$_i$-type subunits, *FEBS Lett.*, 220, 187, 1987.
220. **Kikuchi, A., Kozawa, O., Kaibuchi, K., Katada, T., Ui, M., and Takai, Y.,** Direct evidence for involvement of a guanine nucleotide-binding protein in chemotactic peptide-stimulated formation of inositol bisphosphate and trisphosphate in differentiated human leukemic (HL-60) cells. Reconstitution with G_i or G_o of the plasma membranes ADP-ribosylated by pertussis toxin, *J. Biol. Chem.*, 261, 11558, 1986.
221. **Moriarty, T. M., Gillo, B., Carty, D. J., Premont, R. T., Landau, E. M., and Iyengar, R.,** $\beta\gamma$ subunits of GTP-binding proteins inhibit muscarinic receptor stimulation of phospholipase C, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 8865, 1988.
222. **Birnbaumer, L., Codina, J., Mattera, R., Yatani, A., Scherer, N. M., Toro, M.-J., and Brown, A. M.,** Signal transduction by G proteins, *Kidney Int.*, 32 (Suppl. 23), S14, 1987.
223. **Sugimoto, K., Nukada, T., Tanabe, T., Takahashi, H., Noda, M., Minamino, N., Kangawa, K., Matsuo, H., Hirose, T., Inayama, S., and Numa, S.,** Primary structure of the beta-subunit of bovine transducin deduced from the cDNA sequence, *FEBS Lett.*, 191, 235, 1985.
224. **Codina, J., Stengel, D., Woo, S. L. C., and Birnbaumer, L.,** *Beta* subunits of the human liver G_s/G_i signal transducing proteins and those of bovine retinal rod cell transducin are identical, *FEBS Lett.*, 207, 187, 1986.
225. **Fong, H. K. W., Hurley, J. B., Hopkins, R. S., Miake-Lye, R., Johnson, M. S., Doolittle, R. F., and Simon, M. I.,** Repetitive segmental structure of the transducin beta subunit: homology with the CDC4 gene and identification of related mRNAs, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 2162, 1986.
226. **Fong, H. K. W., Amatruda, T. T., III, Birren, B. W., and Simon, M. I.,** Distinct forms of the β subunit of GTP-binding regulatory proteins identified by molecular cloning, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 3792, 1987.
227. **Gao, B., Gilman, A. G., and Robishaw, J. D.,** A second form of the β subunit of signal-transducing G proteins, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 6122, 1987.
228. **Gao, B., Mumby, S., Gilman, A. G.,** The G protein β_2 complementary DNA encodes the β_{35} subunit, *J. Biol. Chem.*, 262, 17254, 1987.
229. **Levine, M. A., Smallwood, P. M., Moen, P., Helman, L. J., and Modi, W.,** Identification of a cDNA Encoding a third form of the β subunit of GTP-binding regulatory proteins, in *Proceedings of the IXth International Washington Spring Symposium "Biology of Cellular Transducing Signals"*, May 8, 1989.
230. **Amatruda, T. T. III, Gautam, N., Fong, H. K. W., Northup,**

J. K., and Simon, M. I., The 35- and 36-kDa β subunits of GTP-binding regulatory proteins are products of separate genes, *J. Biol. Chem.*, 263, 5008, 1988.

231. **Evans, T., Fawzi, A., Fraser, E. D., Brown, M. L., and Northup, J. K.,** Purification of a $beta_{35}$ form of the *beta-gamma* complex common to G-proteins from human placental membranes, *J. Biol. Chem.*, 262, 176, 1987.
232. **Kühn, H.,** Light-induced, reversible binding of proteins to bovine photoreceptor membranes. Influence of nucleotides, *Neurochemistry*, 1, 269, 1980.
233. **Stryer, L., Hurley, J. B., and Fung, B. K.,** First stage of amplification in the cyclic-nucleotide cascade of vision, *Curr. Top. Membr. Transport*, 15, 93, 1981.
234. **Bitensky, M. W., Wheeler, G. L., Yamazaki, A., Rasenick, M. M., and Stein, P. J.,** Cyclic-nucleotide metabolism in vertebrate photoreceptors: a remarkable analogy and an unravelling enigma, *Curr. Top. Membr. Transport*, 15, 237, 1981.
235. **Hurley, J. B., Fong, H. K. W., Teplow, D. B., Dreyer, W. J., and Simon, M. I.,** Isolation and characterization of a cDNA clone for the gamma subunit of bovine retinal transducin, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 6948, 1984.
236. **Yatsunami, K., Pandya, B. V., Oprian, D. D., and Khorana, H. G.,** cDNA-derived amino acid sequence of the gamma subunit of GTPase from bovine rod outer segments, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 1936, 1985.
237. **Fukada, Y., Ohguro, H., Saito, T., Yoshizawa, T., and Akino, T.** βγ-subunits of bovine transducin composed of two components with distinctive γ-subunits, *J. Biol. Chem.*, 264, 5937, 1989.
238. **Gierschik, P., Codina, J., Simons, C., Birnbaumer, L., and Spiegel, A.,** Antisera against a guanine binding protein from retina cross-react with the *beta* subunit of the adenylyl cyclase associated guanine nucleotide binding proteins, N_s and N_i, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 721, 1985.
239. **Hildebrandt, J. D., Codina, J., Risinger, R., and Birnbaumer, L.,** Identification of a *gamma* subunit associated with the adenylyl cyclase regulatory proteins N_s and N_i, *J. Biol. Chem.*, 259, 2039, 1984.
240. **Hildebrandt, J. D., Codina, J., Rosenthal, W., Birnbaumer, L., Neer, E. J., Yamazaki, A., and Bitensky, M. W.,** Characterization by two-dimensional peptide mapping of the subunit composition of the regulatory N_s and N_i proteins of adenylyl cyclase, and transducin, the guanine nucleotide binding protein of retinal rod outer segments, *J. Biol. Chem.*, 260, 14867, 1985.
241. **Gautam, N., Baetscher, M., Aebersold, R., and Simon, M. I.,** A G protein gamma subunit shares homology with rase proteins, *Science*, 44, 971, 1989.
242. **Litosch, I., Wallis, C., and Fain, J. N.,** 5-Hydroxytryptamine stimulates inositol phosphate production in a cell-free system from blowfly salivary glands. Evidence for a role of GTP in coupling receptor activation to phosphoinositide breakdown, *J. Biol. Chem.*, 260, 5464, 1985.
243. **Cockroft, S. and Gomperts, B. D.,** Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase, *Nature*, 314, 534, 1985.
244. **Paris, S. and Pouysségur, J.,** Pertussis toxin inhibits thrombin-induced activation of phosphoinositide hydrolysis and Na^+/H^+ exchange in hamster fibroblasts, *EMBO J.*, 5, 55, 1986.
245. **Kuroda, M., Honnor, R. C., Cushman, S. W., Londos, C., and Simpson, I.,** Regulation of insulin-stimulated glucose transport in the isolated rat adipocyte. cAMP-independent effects of lipolytic and antipolytic agents, *J. Biol. Chem.*, 262, 245, 1987.
246. **Mallat, A., Pavione, C., Dufour, M., Lotersztajn, S., Bataille, D., and Pecker, F.,** A glucagon fragment is responsible for the inhibition of the liver Ca^{2+} pump by glucagon, *Nature*, 325, 620, 1987.
247. **Bertorello, A. and Aperia, A.,** Regulation of Na^+-K^+-ATPase activity in kidney proximal tubules: involvement of GTP binding proteins, *Am. J. Physiol.*, 256, F57, 1989.
248. **Hughes, A. R., Martin, M. W., and Harden, T. K.,** Pertussis toxin differentiates between two mechanisms of attenuation of cyclic AMP accumulation by muscarinic cholinergic receptors, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 5680, 1984.
249. **Rane, S. O. and Dunlap, K.** Kinase C activator 1,2-oleylacetylglycerol attenuates voltage-dependent calcium current in sensory neurones, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 184, 1986.
250. **Dolphin, A. C. and Scott, R. H.,** Calcium channel currents and their inhibition by (−)-baclofen in rat sensory neurones: modulation by guanine nucleotides, *J. Physiol.*, 386, 1, 1987.
251. **Dolphin, A. C., McGuirk, S. M., and Scott, R. H.,** An investigation into the mechanisms of inhibition of calcium channel currents in cultured rat sensory neurones by guanine nucleotide analogues and (−)-baclofen, *Br. J. Pharmacol.*, 97, 263, 1989.
252. **Perney, T. M. and Miller, R. J.,** Two different G-proteins mediate neuropeptide Y and bradykinin-stimulated phospholipid breakdown in cultured rat sensory neurons, *J. Biol. Chem.*, 264, 7317, 1989.
253. **Cassel, D. and Selinger, Z.,** Catecholamine-stimulated GTPase activity in turkey erythrocyte membranes, *Biochim. Biophys. Acta*, 252, 538, 1976.
254. **Cassel, D. and Selinger, Z.,** Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolysis at the regulatory site, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 3307, 1977.
255. **Godchaux, W., III and Zimmerman, W. F.,** Membrane-dependent guanine nucleotide binding and GTPase activities of soluble protein from bovine rod cell outer segments, *J. Biol. Chem.*, 254, 7874, 1979.
256. **Brandt, D. R., Asano, T., Pedersen, S. E., and Ross, E. M.,** Reconstitution of catecholamine-stimulated guanosinetriphosphatase activity, *Biochemistry*, 22, 4357, 1983.
257. **Cerione, R. A., Codina, J., Benovic, J. L., Lefkowitz, R. J., Birnbaumer, L., and Caron, M. G.,** The mammalian $beta_2$-adrenergic receptor: reconstitution of the pure receptor with the pure stimulatory nucleotide binding protein (N_s) of the adenylate cyclase system, *Biochemistry*, 23, 4519, 1984.
258. **Van Dongen, A. M. J., Birnbaumer, L., and Brown, A. M.,** unpublished results.
259. **Simon, M.,** personal communication.
260. **Codina, J. and Birnbaumer, L.,** unpublished results.
261. **Katani, A., Birnbaumer, L., and Brown, A. M.,** unpublished results.