Inositol Phosphates Modulate Human Red Blood Cell Ca^{2+}–Adenosine Triphosphatase Activity In Vitro by a Guanine Nucleotide Regulatory Protein

Faith B. Davis, Paul J. Davis, Susan D. Blas, and Denise Z. Gombas

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] inhibits human red blood cell (RBC) Ca^{2+}-stimulable, Mg^{2+}-dependent adenosine triphosphatase (Ca^{2+}-ATPase) activity in vitro. Because we have previously shown that adrenergic receptors exist on the human mature RBC membrane and can modulate Ca^{2+}-ATPase activity, we examined the possibility that a guanine nucleotide regulatory protein (G protein) mediated the Ins(1,4,5)P_3 effect. Guanosine 5′-*O*-(3-thiotrisphosphate) (GTPγS) 10^{-4} mol/L also inhibited RBC Ca^{2+}-ATPase activity. Pertussis toxin 200 ng/mL blocked the effects of both Ins(1,4,5)P_3 and GTPγS on Ca^{2+}-ATPase activity. In separate studies, pertussis toxin–catalyzed adenosine diphosphate (ADP) ribosylation was shown to occur in RBC membranes under conditions in which measurements of Ca^{2+}-ATPase activity were performed. When Ins(1,4,5)P_3 10^{-7} mol/L and GTPγS 10^{-6} mol/L were added to membranes concurrently, their inhibitory actions on the enzyme were additive. At greater concentrations of Ins(1,4,5)P_3 (10^{-6} to 10^{-5} mol/L) and GTPγS (10^{-4} mol/L), the inositol phosphate reversed the inhibitory effect of GTPγS. These observations indicate that the novel effect of Ins(1,4,5)P_3 on the activity of a plasma membrane Ca^{2+}-ATPase depends at least in part on the action of a pertussis toxin–susceptible G protein.

HUMAN RED BLOOD CELL (RBC) Ca^{2+}-stimulable, Mg^{2+}-dependent adenosine triphosphatase (Ca^{2+}-ATPase) activity is regulated by the intracellular calmodulin·Ca^{2+} complex[1,2] and several other cellular factors, such as fatty acids,[3,4] phospholipids,[3] and D-glucose.[5,6] We have previously shown that D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and D-*myo*-inositol 4,5-bisphosphate, but not other inositol phosphates inhibit RBC Ca^{2+}-ATPase in vitro.[7] This effect of a product [Ins(1,4,5)P_3] of the phosphoinositide pathway is one of a series of recent observations supporting plasma membrane actions of inositol phosphates[8-10] distinct from the Ca^{2+}-liberating effects of the compounds on Ins(1,4,5)P_3-sensitive intracellular Ca^{2+} pools.[11]

Our laboratory has reported that Ins(1,4,5)P_3 can inhibit the binding of calmodulin, a major cytoplasmic Ca^{2+}-binding protein,[1,2] to the human RBC membrane,[7] and this may be one mechanism by which this inositol phosphate can affect Ca^{2+}-ATPase activity. Ins(1,4,5)P_3 also antagonizes the binding of other small molecules, such as thyroid hormone, to RBC ghosts.[12] On the other hand, Ins(1,4,5)P_3 can reduce Ca^{2+}-ATPase activity in the absence of added purified calmodulin,[7] and this observation suggested that inositol phosphates may affect the enzyme by a mechanism separate from that involving the calmodulin·Ca^{2+} complex. Because we have shown that α_1- and β-adrenergic receptors are present in the human RBC membrane and that ligands for such receptors can increase or decrease the activity of membrane Ca^{2+}-ATPase[13] depending on experimental conditions, we proceeded in the current studies to examine erythrocyte ghosts for evidence of the presence of a guanine nucleotide regulatory protein (G protein) that may affect Ca^{2+}-ATPase activity.

SUBJECTS AND METHODS

Reagents

Ins(1,4,5)P_3 and pertussis toxin were obtained from Calbiochem (San Diego, CA), Na_2 adenosine triphosphate (Na_2ATP) and guanosine 5′-*O*-(3-thiotrisphosphate) (GTPγS) from Sigma (St Louis, MO), and [^{32}P]NAD from New England Nuclear (Boston, MA).

Human Erythrocyte Membranes

Heparinized venous blood was obtained from healthy adult volunteers under a protocol approved by the Institutional Review Boards and after provision of informed consent. Membranes were prepared as previously described,[14] by hypotonic lysis of cells in 10 mmol/L Tris, pH 7.45, and 0.1 mmol/L EDTA. Harvested membranes were washed in 10 mmol/L Tris, pH 7.45, and stored at −70°C until used in the Ca^{2+}-ATPase assay within 5 days.

Ca^{2+}-ATPase Activity

Enzyme activity was measured as the difference in hydrolysis of Na_2ATP in the presence and absence of 2×10^{-5} mol/L free Ca^{2+} as previously reported,[15] and expressed as micromoles P_i liberated per milligram membrane protein after a 90-minute assay period. The assay buffer contained 25 mmol/L Tris, pH 7.45, 0.1 mmol/L EGTA, 75 mmol/L NaCl, 25 mmol/L KCl, 1 mmol/L $MgCl_2$, and 1 mmol/L Na_2ATP, with or without 0.15 mmol/L $CaCl_2$. P_i level was measured by the Fiske-Subbarow method[16] and membrane protein level by the Lowry method,[17] with bovine serum albumin as standard. Results presented are the mean ± SE of at least three experiments conducted in duplicate.

Effect of Ins(1,4,5)P_3, GTPγS, and Pertussis Toxin on Ca^{2+}-ATPase Activity

Actions of these substances were determined by adding each individually or concurrently in the concentrations specified to aliquots of RBC membranes (1.0 mg/mL) and incubating the resultant mixtures for 60 minutes at 37°C before enzyme assay. Control samples contained the diluent for each substance, which was 10 mmol/L Tris, pH 7.45. After this preincubation, aliquots of

From the Division of Molecular and Cellular Medicine, Department of Medicine, Albany Medical College, Albany; Stratton Department of Veterans Affairs Medical Center, Albany; and Department of Medicine, State University of New York at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY.

Submitted February 7, 1994; accepted November 9, 1994.

Supported in part by Department of Veterans Affairs Merit Review funding (P.J.D.).

Address reprint requests to Paul J. Davis, MD, Professor of Medicine, Department of Medicine A-57, Albany Medical College, Albany, NY 12208.

0026-0495/95/4407-0006$03.00/0

each sample were incubated in the assay buffer in the presence or absence of Ca^{2+}, as described earlier.

Effect of Pertussis Toxin on Adenosine Diphosphate Ribosylation of a 39-kd RBC Membrane Protein

In phase 1 of these experiments, RBC membranes were incubated with or without pertussis toxin 200 ng/mL in 10 mmol/L Tris, pH 7.45, for 60 minutes at 37°C, followed by a 90-minute incubation in Ca^{2+}-ATPase assay buffer containing Ca^{2+}, thus using our standard preincubation and assay protocol. The membranes were then centrifuged at 14,000 × *g* for 2 minutes, and the membrane pellet was resuspended in 2% Triton X-100 containing 4 μg/mL aprotinin and 1 μg/mL leupeptin. The membranes were then subjected to phase 2 pertussis toxin–catalyzed adenosine diphosphate (ADP) ribosylation using [^{32}P]NAD, based on a modification of the method reported by Ribeiro et al.[18] The resuspended membranes were diluted (final concentration, 100 μg protein/30 μL) with buffer containing (in final concentrations) 2 mmol/L Tris, pH 7.6, 2 mmol/L EDTA, 2 mmol/L dithiothreitol (DTT), 2 mmol/L Na_2ATP, and 25 μg/mL pertussis toxin. After addition of 0.5 μCi [^{32}P]NAD, the samples were incubated at 32°C for 20 minutes. The reaction was stopped with Laemmli solubilizer,[19] and the proteins in each sample were separated by discontinuous 12% polyacrylamide gel electrophoresis, followed by gel drying and radioautography.

RESULTS

Inhibition of Ca^{2+}-ATPase Activity by GTPγS

At concentrations of 10^{-6} and 10^{-4} mol/L, GTPγS reduced enzyme activity by 12% and 35%, respectively ($P < .001$, one-way ANOVA; Fig 1). In the presence of these concentrations of GTPγS, 10^{-7} mol/L Ins(1,4,5)P_3 had a further inhibitory effect on Ca^{2+}-ATPase activity, whereas higher concentrations of Ins(1,4,5)P_3 (10^{-6} to 10^{-5} mol/L) blocked the inhibitory effect of GTPγS (Fig 1). The effects of GTP 10^{-6} to 10^{-4} mol/L were similar to those of GTPγS (results not shown).

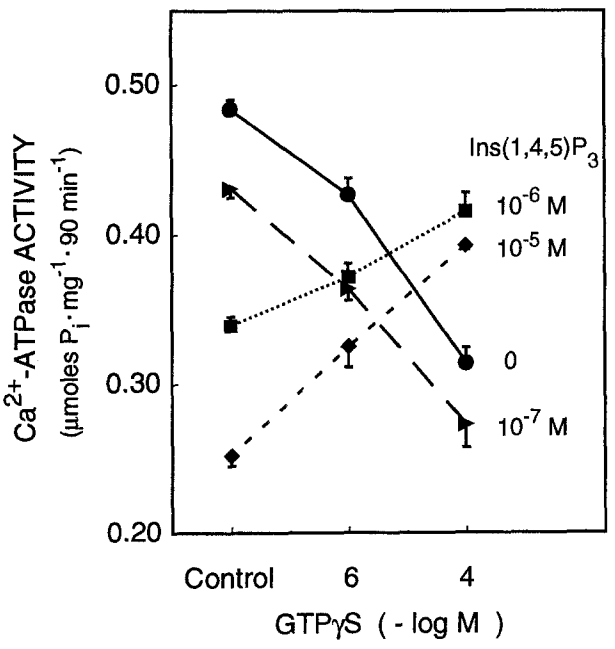

Fig 1. Effect of GTPγS and Ins(1,4,5)P_3 on human RBC Ca^{2+}-ATPase activity. GTPγS 10^{-6} and 10^{-4} mol/L progressively inhibited enzyme activity (●), as did Ins(1,4,5)P_3 10^{-7} to 10^{-5} mol/L (control values at left). The inhibitory effect of Ins(1,4,5)P_3 10^{-7} mol/L (▶) was additive to that of GTPγS, whereas Ins(1,4,5)P_3 10^{-6} (■) and 10^{-5} (◆) mol/L blocked the inhibitory effect of 10^{-4} mol/L GTPγS on the enzyme.

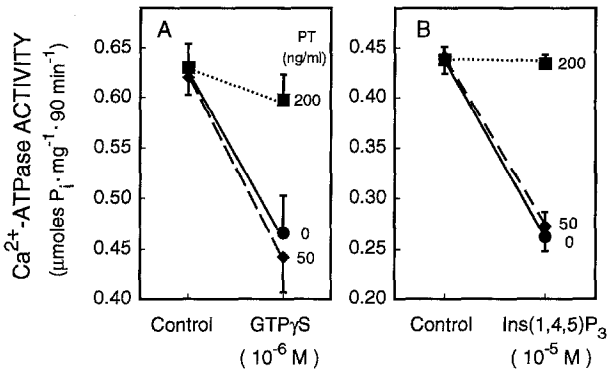

Fig 2. Effect of pertussis toxin (PT) on inhibition of RBC Ca^{2+}-ATPase activity by GTPγS (A) and Ins(1,4,5)P_3 (B). PT 50 and 200 ng/mL had no effect alone on enzyme activity (control values in each panel). There was complete inhibition of GTPγS and Ins(1,4,5)P_3 effects by 200 ng/mL pertussis toxin (■), but no inhibition by 50 ng/mL pertussis toxin (◆). Differences in absolute levels of control enzyme activity in A and B are due to different RBC membrane preparations used in the two sets of experiments.

Action of Ins(1,4,5)P_3 on Ca^{2+}-ATPase Activity

As previously shown[7] and as evident in Fig 1, Ins(1,4,5)P_3 10^{-7} to 10^{-5} mol/L decreased human RBC Ca^{2+}-ATPase activity significantly ($P < .001$, ANOVA). GTPγS concentrations of 10^{-6} and 10^{-4} mol/L blocked the inhibitory effect of 10^{-6} and 10^{-5} mol/L Ins(1,4,5)P_3 on the enzyme.

Effect of Pertussis Toxin on Enzyme Activity

Pertussis toxin (50 to 200 ng/mL) added to RBC membranes had no significant effect alone on Ca^{2+}-ATPase activity (Fig 2A and B control values). The toxin at a concentration of 50 ng/mL had no effect on the inhibitory action of GTPγS (Fig 2A) or Ins(1,4,5)P_3 (Fig 2B) on Ca^{2+}-ATPase activity, but at 200 ng/mL, pertussis toxin completely reversed the inhibitory action of both agents. However, the higher concentration of pertussis toxin did not block the inhibitory effect of 10^{-4} mol/L GTPγS (results not shown).

Effect of Pertussis Toxin on ADP Ribosylation of RBC Membrane Protein

Measurement of ADP ribosylation in membranes previously exposed in phase 1 to 200 ng/mL pertussis toxin allowed us to assay previously unused ribosylation sites, demonstrated by radiolabeling in the presence of [^{32}P]NAD and an excess of pertussis toxin activated by DTT (phase 2), using the method reported by Ribeiro et al.[18] In Fig 3, lane 1, there was no labeling of membranes that had not been exposed to pertussis toxin either in phase 1 or in phase 2. In lane 2, in membranes not exposed to pertussis toxin in phase 1 but later exposed to pertussis toxin, DTT, and radiolabeled NAD in phase 2, there was ample labeling of a

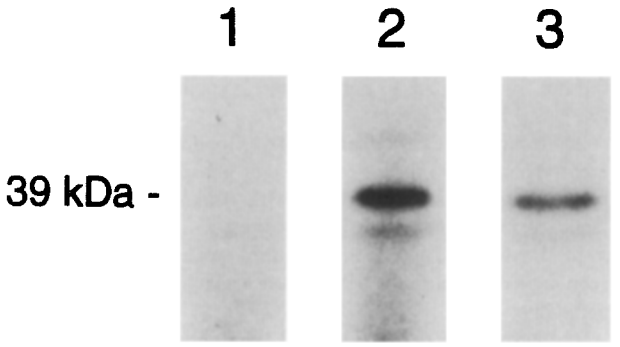

Fig 3. Effect of prior exposure of RBC membranes to pertussis toxin (phase 1) on subsequent [^{32}P]ADP ribosylation of a 39-kd membrane protein band on a second exposure to pertussis toxin (phase 2). Sample 1 was not exposed to pertussis toxin in either phase. Sample 2 was only exposed to pertussis toxin in phase 2, when DTT was also present. Sample 3 was exposed to pertussis toxin in phase 1, causing ADP ribosylation at that time and reducing substrate availability for labeling during phase 2.

39-kd band, consistent with the results reported by Ribeiro et al.[18] Lane 3 demonstrates decreased radiolabeling in a sample exposed to pertussis toxin in both phases, providing evidence that during the 60-minute preincubation and 90-minute assay periods ADP ribosylation did occur in RBC membrane samples in the presence of pertussis toxin 200 ng/mL, yielding less available substrate for labeling when the membranes were later exposed to an excess of DTT-activated pertussis toxin and radiolabeled NAD.

DISCUSSION

The calmodulin•Ca^{2+} complex[1,2] and lipids[3] in the plasma membrane microenvironment of erythrocyte Ca^{2+}-ATPase are intrinsic factors known to affect the activity of the enzyme. We have also shown that Ins(1,4,5)P_3, a product of the action of phospholipase C in the phosphoinositide pathway,[11,20] inhibits Ca^{2+}-ATPase activity at a concentration known to be physiologic.[7] It has been established that phospholipase C is present in RBC membranes, and that in the presence of added calcium, membrane content of inositol phosphates increases.[21-23] Absolute levels of inositol phosphates in whole RBCs or RBC membranes have not been reported. A number of extracellular factors also affect Ca^{2+}-ATPase activity,[24] including thyroid hormone[14,15,25] and D-glucose,[5,6] and in myocardial and skeletal muscle membranes, several pharmacologic agents.[26-29] The mechanisms by which these factors act to modulate Ca^{2+}-ATPase activity are incompletely understood, although several of the factors bind to calmodulin and may disrupt the interaction of the enzyme and the calmodulin•Ca^{2+} complex.[27-29]

Adrenergic agonists and polypeptide hormones such as glucagon depend on cell-surface receptors specific for the hormone ligands and subsequent signal transduction by guanine nucleotide regulatory proteins (G proteins).[30] The turkey erythrocyte has been extensively studied as a model of the hormone receptor–G protein complex.[31-33] The presence of GTP-binding proteins in human erythrocyte membranes has also been reported.[33-35] No physiologic role for these proteins in the RBC has previously been established,[36] although changes in a stimulatory G protein (G_s) extracted from human erythrocytes and identified as stimulatory in a mouse cell line adenylate cyclase system have been used as a marker for pseudohypoparathyroidism.[37] The current report provides functional significance for a G protein (inhibitory G protein [G_i], recognized by its susceptibility to pertussis toxin) in human RBC membranes in the regulation of Ca^{2+}-ATPase activity.

The lines of evidence presented here for the existence of G proteins that are functional in the human erythrocyte ghost are (1) the ability of GTPγS and GTP to inhibit erythrocyte Ca^{2+}-ATPase activity and (2) the ability of pertussis toxin, an agent that inactivates inhibitory guanine nucleotide regulatory proteins (G_i), to antagonize the effects of GTPγS on Ca^{2+}-ATPase. We also show that the inhibitory action of Ins(1,4,5)P_3 on RBC Ca^{2+}-ATPase activity can be blocked by pertussis toxin and by GTPγS when both factors are used in maximal concentrations, suggesting that the inositol phosphate and GTPγS may both act on a G_i protein in this model, and when present together, may act at related sites on G_i and result in an inactivated protein, perhaps by an allosteric mechanism. The fact that the susceptibility to pertussis toxin of the Ins(1,4,5)P_3 effect on membrane Ca^{2+}-ATPase activity indeed reflected participation of a G protein was confirmed by additional studies showing the presence in RBC membranes of pertussis toxin–catalyzed ADP ribosylation. It was necessary to prove that ADP ribosylation can occur in erythrocyte membranes in which Ca^{2+}-ATPase activity measurements are made, since such membranes are exposed to Mg^{2+} and phosphate, factors known to reduce ADP ribosylation.[38] The additive effect of submaximal concentrations of Ins(1,4,5)P_3 and GTPγS on inhibition of Ca^{2+}-ATPase activity can also be explained: GTPγS may stimulate phospholipase C by a G protein–dependent mechanism, leading to increased Ins(1,4,5)P_3 concentrations and further inhibition of Ca^{2+}-ATPase activity. These observations support our contention[7] that the activity of the phosphoinositide pathway is a determinant of the activity of Ca^{2+}-ATPase in the human RBC.

The concept that the calmodulin•Ca^{2+} complex is the principal regulator of human RBC Ca^{2+}-ATPase activity[1,2] is confining in light of the substantial number of extracellular factors that may modulate activity of the enzyme.[24] The fact that mature RBC Ca^{2+}-ATPase activity can be modulated by G proteins indicates that mechanisms may exist by which extracellular hormones, such as those traditionally acknowledged to act via surface receptor–linked G proteins, may modulate behavior of the enzyme and presumably intracellular Ca^{2+} concentration. G proteins have not previously been implicated in the regulation of human RBC Ca^{2+}-ATPase activity. The presence of adrenergic receptors[13] and G proteins in the human mature RBC membrane that are capable of regulating membrane Ca^{2+}-ATPase (calcium pump) activity suggests that this human cell system may be used to model adrenergic activity in vitro. There is crosstalk between the G protein and inositol phosphate signaling pathways in this model.

REFERENCES

1. Scharff O: Calmodulin and its role in cellular activation. Cell Calcium 2:1-27, 1981
2. James P, Maeda M, Fischer R, et al: Identification and primary structure of a calmodulin binding domain of the Ca^{2+} pump of human erythrocytes. J Biol Chem 263:2905-2910, 1988
3. Niggli V, Adunyah ES, Carafoli E: Acidic phospholipids, unsaturated fatty acids, and limited proteolysis mimic the effect of calmodulin on the purified erythrocyte Ca^{2+}-ATPase. J Biol Chem 256:8588-8592, 1981
4. Davis FB, Davis PJ, Blas SD, et al: Action of long-chain fatty acids in vitro on Ca^{2+}-stimulatable, Mg^{2+}-dependent ATPase activity in human red cell membranes. Biochem J 248:511-516, 1987
5. Davis FB, Davis PJ, Nat G, et al: The effect of in vivo glucose administration on human erythrocyte Ca^{2+}-ATPase activity and on enzyme responsiveness in vitro to thyroid hormone and calmodulin. Diabetes 34:639-646, 1985
6. Deziel MR, Safeer RS, Blas SD, et al: Hexose-specific inhibition in vitro of human red cell Ca^{2+}-ATPase activity. Biochim Biophys Acta 1110:119-122, 1992
7. Davis FB, Davis PJ, Lawrence WD, et al: Specific inositol phosphates inhibit basal and calmodulin-stimulated Ca^{2+}-ATPase activity in human erythrocyte membranes in vitro and inhibit binding of calmodulin to membranes. FASEB J 5:2992-2995, 1991
8. Kuno N, Gardner P: Ion channels activated by inositol 1,4,5-trisphosphate in plasma membrane of human T-lymphocytes. Nature 326:301-304, 1987
9. Penner R, Matthews G, Neher E: Regulation of calcium influx by second messengers in rat mast cells. Nature 334:499-504, 1988
10. Mayr GW: Inositol 1,4-bisphosphate is an allosteric activator of muscle-type 6-phospho-fructo-1-kinase. Biochem J 259:463-470, 1989
11. Berridge MJ: Temporal aspects of calcium signalling, in Nishizuka Y, Endo M, Tanaka C (eds): The Biology and Medicine of Signal Transduction. New York, NY, Raven, 1990, pp 108-114
12. Davis FB, Moffett MJ, Davis PJ, et al: Inositol phosphates modulate binding of thyroid hormone to human red cell membranes in vitro. J Clin Endocrinol Metab 77:1427-1430, 1993
13. Sundquist J, Blas SD, Hogan JE, et al: The α_1-adrenergic receptor in human erythrocyte membranes mediates interaction in vitro of epinephrine and thyroid hormone at the membrane Ca^{2+}-ATPase. Cell Signal 4:795-799, 1992
14. Davis PJ, Blas SD: In vitro stimulation of human red blood cell Ca^{2+}-ATPase by thyroid hormone. Biochem Biophys Res Commun 99:1073-1080, 1981
15. Davis FB, Davis PJ, Blas SD: Role of calmodulin in thyroid hormone stimulation in vitro of human erythrocyte Ca^{2+}-ATPase activity. J Clin Invest 71:579-586, 1983
16. Fiske CH, Subbarow Y: The colorimetric determination of phosphorus. J Biol Chem 66:375-400, 1925
17. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951
18. Ribeiro CMP, Dubay GR, Falck JR, et al: Parathyroid hormone inhibits Na^+-K^+-ATPase through a cytochrome P-450 pathway. Am J Physiol 266:F497-F505, 1994
19. Laemmli U: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685, 1970
20. Majerus PW, Ross TS, Cunningham TW, et al: Recent insights in phosphatidylinositol signaling. Cell 63:459-465, 1990
21. Downes CP, Michell RH: The polyphosphoinositide phosphodiesterase of erythrocyte membrane. Biochem J 198:133-140, 1981
22. Tremblay J, Cherkaoui L, Skuherska R, et al: Increased inositol trisphosphate in erythrocytes of spontaneously hypertensive rats. J Hypertens 8:115-119, 1990
23. Doughney C, McPherson MA, Goodchild MC, et al: Increased phosphoinositide breakdown by phospholipase C in erythrocyte membranes from patients with cystic fibrosis. Clin Chim Acta 181:55-64, 1989
24. Davis PJ, Davis FB, Lawrence WD: Thyroid hormone regulation of membrane Ca^{2+}-ATPase activity. Endocr Res 15:651-682, 1989
25. Davis FB, Cody V, Davis PJ, et al: Stimulation by thyroid hormone analogues of red blood cell Ca^{2+}-ATPase activity in vitro. J Biol Chem 258:12373-12377, 1983
26. Mylotte KM, Cody V, Davis PJ, et al: Milrinone and thyroid hormone stimulate myocardial membrane Ca^{2+}-ATPase activity and share structural homologies. Proc Natl Acad Sci USA 82:7974-7978, 1985
27. Warnick PR, Davis FB, Davis PJ, et al: Differential activities of tolbutamide, tolazamide, and glyburide in vitro on rabbit myocardial membrane Ca^{2+}-transporting ATPase activity. Diabetes 35:1044-1048, 1986
28. Warnick PR, Davis FB, Mylotte KM, et al: Calcium channel blocker inhibition of the calmodulin-dependent effects of thyroid hormone and milrinone on rabbit myocardial membrane Ca^{2+}-ATPase activity. Biochem Pharmacol 37:2619-2623, 1988
29. Deziel MR, Davis PJ, Davis FB, et al: Interaction of amiodarone and its analogs with calmodulin. Arch Biochem Biophys 274:463-470, 1989
30. Freissmuth M, Casey PJ, Gilman AG: G proteins control diverse pathways of transmembrane signaling. FASEB J 3:2125-2131, 1989
31. Harden TK, Stephens L, Hawkins PT, et al: Turkey erythrocyte membranes as a model for regulation of phospholipase C by guanine nucleotides. J Biol Chem 262:9057-9061, 1987
32. Vaziri C, Downes CP: Association of a receptor and G-protein–regulated phospholipase C with the cytoskeleton. J Biol Chem 267:22973-22981, 1992
33. English D, Akard LP, Taylor GS, et al: G_p-regulated phosphoinositide hydrolysis in turkey and human erythrocytes exposed to fluoride ion: Relationship to calcium influx. J Lab Clin Med 119:87-98, 1992
34. Iyengar R, Rich KA, Herberg JT, et al: Identification of a new GTP-binding protein. J Biol Chem 262:9239-9245, 1987
35. Carty DJ, Padrell E, Codina J, et al: Distinct guanine nucleotide binding and release properties of the three G_i proteins. J Biol Chem 265:6268-6273, 1990
36. DeFlora A, Damonte G, Sdraffa A, et al: Heterogeneity of guanine nucleotide binding proteins in human red blood cell membranes. Adv Exp Med Biol 307:161-171, 1991
37. Levine MA, Jap TS, Mauseth RS, et al: Activity of the stimulatory guanine nucleotide–binding protein is reduced in erythrocytes from patients with pseudohypoparathyroidism and pseudopseudohypoparathyroidism: Biochemical, endocrine and genetic analysis of Albright's hereditary osteodystrophy in six kindreds. J Clin Endocrinol Metab 62:497-502, 1986
38. Ribeiro-Neto F, Mattera R, Grenet D, et al: Adenosine diphosphate ribosylation of G proteins by pertussis and cholera toxin in isolated membranes. Different requirements for and effects of guanine nucleotides and Mg^{2+}. Mol Endocrinol 1:472-481, 1987