Cyclic GMP (cGMP) inhibits intracellular calcium ((Ca^{2+})_{i}) mobilization in vascular smooth muscle cells by a mechanism that is not well understood. Because several studies suggest that cGMP inhibits inositol 1,4,5-trisphosphate (IP_{3}) action, we examined the effects of cGMP-dependent protein kinase on IP_{3} receptor phosphorylation. The purified IP_{3} receptor was phosphorylated using either the cGMP, or cAMP-dependent protein kinase in vitro. Phosphorylation was time-dependent and stoichiometric using both kinases. Two-dimensional phosphopeptide mapping, high performance liquid chromatography analysis, and amino acid analysis showed that identical sites were phosphorylated using either kinase, and identified serine 1755 as the site of phosphorylation. The synthetic peptide corresponding to serine 1755 (GRRESLTSFG) was phosphorylated with a K_{m} in the range of 30-40 μM by both kinases. The kinetic analysis revealed that this peptide substrate is the best substrate described for cGMP kinase to date. Vascular smooth muscle cells prelabeled with [32P]orthophosphate and treated with atrial natriuretic peptide or sodium nitroprusside to elevate cGMP also resulted in phosphorylation of the IP_{3} receptor. Phosphorylation of IP_{3} receptor by cGMP kinase may regulate the function of IP_{3} receptor in vascular smooth muscle cells and contribute to the effect of cGMP to regulate intracellular calcium levels.

Cyclic GMP mediates vascular smooth muscle relaxation evoked by nitrovasodilators, nitric oxide, and atrial natriuretic peptides (1–6). Although the precise mechanisms by which cGMP causes vascular smooth muscle relaxation are not well understood, studies have shown that the effects of cGMP are mediated through cGMP kinase and that the reduction of cytoplasmic [Ca^{2+}]_{i}, evoked by cGMP kinase in vascular smooth muscle cells leads to relaxation (7–9). Several mechanisms have been proposed to account for cGMP-dependent [Ca^{2+}]_{i} reduction including activation of Ca^{2+}-ATPase in the plasma membrane and sarcoplasmic reticulum (10–12), activation of Ca^{2+}-activated K⁺ channels (13–15), and inhibition of inositol 1,4,5-trisphosphate (IP_{3}) formation (16, 17). Each of these mechanisms probably plays an important role in relaxation of several vascular smooth muscle preparations. Other studies have indicated that cGMP inhibits agonist-evoked Ca^{2+} release presumably by inhibiting the effects of IP_{3} on the sarcoplasmic reticulum (18–20). If inhibition of IP_{3}-induced Ca^{2+} release is one mechanism by which cGMP contributes to the inhibition of contractions due to agonists in vascular tissues, it is possible that cGMP kinase catalyzes the phosphorylation of the IP_{3} receptor.

The IP_{3} receptor has been purified from mouse (21) and rat cerebella (22) and has been reconstituted into liposomes and shown to release Ca^{2+} as a function of ligand binding (23). Although several forms of the IP_{3} receptor have been identified (24), the protein isolated and characterized from both smooth muscle (25) and brain (26) appear to be structurally and functionally identical. The purified IP_{3} receptor from smooth muscle formed an IP_{3}-gated and heparin-sensitive Ca^{2+} channel in planar bilayers (27). Supattapone et al. (28) demonstrated that the purified IP_{3} receptor from cerebellum was stoichiometrically phosphorylated by cAMP kinase and that phosphorylation resulted in diminished potency of IP_{3} in releasing Ca^{2+} from brain membrane fractions. Both protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II also catalyzed phosphorylation of the IP_{3} receptor in reconstituted lipid vesicles (29).

Because of the potential role for cGMP and cGMP kinase in the regulation of Ca^{2+} signaling in smooth muscle, we examine the effects of cGMP kinase on IP_{3} receptor phosphorylation in vitro and in intact rat aortic smooth muscle cells. Our results indicate that IP_{3} receptor phosphorylation may be one mechanism by which cGMP regulates [Ca^{2+}]_{i} in vascular smooth muscle.

EXPERIMENTAL PROCEDURES

Materials—The peptides GRRESLTSFG and ARRDSVLAAS were purchased from Research Genetics (Huntsville, AL). Heparin-agarose and concanavalin A-Sepharose, protease inhibitors, and other chemicals were purchased from Sigma. [γ-32P]ATP and [32P]orthophosphoric acid were obtained from DuPont NEN. Protein kinase inhibitor peptide (5–22) was obtained from Peninsula Laboratories.

Purification and Phosphorylation of the IP_{3} Receptor—The IP_{3} receptor was purified from rat cerebellum according to Supattapone et al. (28) except that protease inhibitors, 0.1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and 10 μM pepstatin A were added to all buffers in the purification steps and 2-mercaptoethanol was increased from 1 to 10 mM. For in vitro phosphorylation studies, 10 μM of the purified receptor was incubated with 10 μl of either 75 nM cGMP kinase in 20 mM potassium phosphate, pH 7.0, 2 mM EDTA, 150 mM NaCl, 15 mM 2-mercaptoethanol (PEM buffer) containing 1 mg/ml bovine serum albumin and 1 μM cGMP or 150 nM catalytic subunit of cAMP kinase in PEM buffer containing 1 mg/ml bovine serum albumin at 30 °C. Phosphorylation was initiated with the addition of a standard phosphorylation mixture (20 μl) consisting of 40 mM Tris, pH 7.4, 20 mM magnesium acetate, 100 μM 3-isobutyl-1-methylxanthine, and 200 μM [γ-32P]ATP (approximately 150 cpm/μmol). For cGMP kinase reactions, 0.9 μM protein...
Phosphorylation of IP$_3$ Receptor by cGMP Kinase

kinase inhibitor peptide (5-22) was added to the phosphorylation mixture. Incubations were carried out for different times, and reactions were terminated with the addition of 10 μl of an electrophoresis stop mix (sample buffer) containing 10% SDS, 2.5 μL 2-mercaptoethanol, 20% glycerol, and 0.1% bromphenol blue. The mixture was heated at 90 °C for 3 min, and denatured proteins were resolved by SDS-PAGE followed by silver staining. The IP$_3$ receptor isoelectrically focused by non-dimensional Phosphopeptide Mapping—The tryptic digests of the IP$_3$ receptor phosphorylated by cGMP kinase or CAMP kinase were separated by a two-dimensional procedure using high voltage electrophoresis (Multiphor II Electrophoresis system) in the first dimension and ascending chromatography in the second dimension (33). The phosphopeptides were redissolved in 30 μl of high voltage electrophoresis buffer (formic acid/acetic acid/pyridine/water, 5:0.5:94.5, pH 3.5). “P-labeled phosphopeptides were detected by autoradiography.

Phosphorylation of the Peptides GRESSLSFG and ARRSVS-LAAS—The peptide solutions of different concentrations (5–500 μM) were incubated with the phosphorylation mixture described earlier at 30 °C. Phosphorylation was initiated by adding cGMP kinase or catalytic subunit of cAMP kinase in a total reaction volume of 70 μl with final concentration of cGMP kinase and cAMP kinase of 21 and 42 μg, respectively. After 5 min, a 50-μl aliquot was removed and spotted on phosphocellulose filter paper (1 x 1 cm) and dropped into a beaker of 75 μmophosphoric acid to stop the reaction. The paper was washed with a second 5% phosphoric acid wash and dried, and subjected to autoradiography at -80 °C.

The time course of phosphorylation of the IP$_3$ receptor was performed as follows: 35 μl of purified IP$_3$ receptor was incubated at 30 °C with the phosphorylation mixture (70 μl). The reaction was initiated by adding 35 μl of either cGMP kinase in PEM buffer containing 1 mg/ml bovine serum albumin and 1 μM cGMP or catalytic subunit of cAMP kinase in PEM buffer with 1 mg/ml bovine serum albumin. The final concentrations of cGMP kinase and cAMP kinase were 30 and 60 μg, respectively, and the IP$_3$ receptor was 375 μg. Incubations were carried out for different time intervals. An aliquot of the reaction mixture (20 μl) was spotted on 3 MM Whatman filter paper and was dried and subjected to autoradiography at -80 °C.

The stoichiometry of phosphorylation was assessed from the specific activity of $^{32}$PiATP and from the amount of IP$_3$ receptor used.

Isolation and Sequencing of Phosphopeptides—Phosphopeptides from the purified cerebellar IP$_3$ receptor were prepared as follows: the purified IP$_3$ receptor (~100 μg) was phosphorylated in a medium containing 40 μM Tris, pH 7.4, 20 mM magnesium acetate, and 200 μM $^{32}$PiATP (Perkin-Elmer RP/5 micron, 15 cm x 4.6 mm) and eluted by a gradient of acetonitrile.

The time course of phosphorylation of the IP$_3$ receptor was carried out by adding 35 μl of either cGMP kinase or CAMP kinase of 21 and 42 μg, respectively, 25 μM tryptic kinase inhibitor peptide (5-22) was added to the phosphorylation mixture at 30 °C. The IP$_3$ receptor was incubated with the phosphorylation mixture described earlier at 30 °C. After incubation, the samples were subjected to electrophoresis on 7.5% SDS-polyacrylamide gels. The gels were stained with Coomassie Blue, dried, and descending chromatography in the second dimension (33). The phosphopeptides were detected by autoradiography.

The phosphorylation was initiated by adding cGMP kinase or catalytic subunit of cAMP kinase in a total reaction volume of 70 μl with final concentration of cGMP kinase and cAMP kinase of 21 and 42 μg, respectively. After 5 min, a 50-μl aliquot was removed and spotted on phosphocellulose filter paper (1 x 1 cm) and dropped into a beaker of 75 μmophosphoric acid to stop the reaction. The mixture was washed with a second 5% phosphoric acid wash and dried, and subjected to autoradiography at -80 °C.

Radioactive fractions were lyophilized and applied to a second HPLC graphed on the same column to ensure purity. The phosphopeptide was labeled phosphoamino acids were detected by autoradiography.

Two-dimensional Phosphopeptide Mapping—The tryptic digests of the IP$_3$ receptor phosphorylated by cGMP kinase or CAMP kinase were separated by a two-dimensional procedure using high voltage electrophoresis (Multiphor II Electrophoresis system) in the first dimension and ascending chromatography in the second dimension (33). The phosphopeptides were redissolved in 30 μl of high voltage electrophoresis buffer (formic acid/acetic acid/pyridine/water, 5:0.5:94.5, pH 3.5) supplemented with 0.1% phenylmethylsulfonyl fluoride, 0.1% Triton X-100, 0.1 mM NaCl, and 0.1% phenylmethlysulfonyl fluoride, 0.1% leupeptin, 10 μM pepstatin A, and 10 μM leupeptin mixed. The digests obtained from the phosphorylated IP$_3$ receptor by cGMP kinase were prepared as follows: the tryptic digest of the IP$_3$ receptor was incubated with 10 mM NEM, 10 μM EDTA, 10 μM NaF, and 10 μM NaCl. The tryptic digest was washed 3 times with 250 μl of buffer A (50 mM Tris-HCl, pH 7.7, 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1 mM phenylmethlysulfonyl fluoride, 10 μM pepstatin A, 10 μM leupeptin, 1 μM okadaic acid) containing 1% Triton X-100 and 0.25 mM NaCl and incubated on ice for 30 min to solubilize the IP$_3$ receptor.

The suspension was centrifuged at 12,000 x g for 30 min. Phosphorylated IP$_3$ receptor was isolated by using the protocol described previously (34). The supernatant was combined with 50 μl of buffer B containing 0.5 M NaCl, and the gels were dried and counted with 10 ml of scintillation cocktail in a scintillation counter. The time course of phosphorylation, the peptides (50 μg) were incubated with cGMP kinase (30 μM) or catalytic subunit of cAMP kinase (60 μM) for different time intervals. An aliquot of the phosphorylated peptide mixture was spotted on phosphocellulose paper and processed as described earlier. The amount of phosphoamino acids was assessed from the specific activity of $^{32}$PiATP and from the amount of peptides used.

Phosphorylation of IP$_3$ Receptor in Primary Culture of Vascular Smooth Muscle Cells—Rat aortic smooth muscle cells were isolated using the procedure of Smith and Brock (34) with modifications as described previously (8). Cells from rat aortas were placed in 6 x 60-mm tissue culture plates and grown to confluence (6–8 days). Cultures were then serum-deprived for 48 h before the experiment. The monolayers were washed in solution containing 0.1% bovine serum albumin buffered with Tris to pH 7.4, 140 mM NaCl, 4.5 mM KCl, and 1.5 mM CaCl$_2$ and were incubated in 3 ml of balanced salt solution containing 0.25 mM cAMP and 0.1% P-labeled orthophosphoric acid for 2 h at 37 °C in an air incubator. After labeling, the cells were rinsed twice with balanced salt solution and incubated with 0.1 μM atrial natriuretic peptide or sodium nitroprusside for 3 min. The cultures were washed three times with 500 μl of buffer A (50 mM Tris-HCl, pH 7.7, 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.1 mM phenylmethlysulfonyl fluoride, 10 μM pepstatin A, 10 μM leupeptin, 1 μM okadaic acid) containing 1% Triton X-100 and 0.25 mM NaCl and incubated on ice for 30 min to solubilize the IP$_3$ receptor.

The suspension was centrifuged at 12,000 x g for 30 min. Phosphorylated IP$_3$ receptor was isolated by using the protocol described previously (34). The IP$_3$ receptor phosphorylated by cGMP kinase or CAMP kinase was separated by a two-dimensional procedure using high voltage electrophoresis (Multiphor II Electrophoresis system) in the first dimension and ascending chromatography in the second dimension (33). The phosphopeptides were redissolved in 30 μl of high voltage electrophoresis buffer (formic acid/acetic acid/pyridine/water, 5:0.5:94.5, pH 3.5). “P-labeled phosphopeptides were detected by autoradiography.
RESULTS

Purification of the IP₃ Receptor—The IP₃ receptor was purified from freshly collected rat cerebellum as described under "Experimental Procedures." The purified receptor was analyzed by 7.5% SDS-PAGE and shown to contain a single polypeptide band migrating at ~260 kDa (Fig. 1). Silver staining and copper staining (Bio-Rad kit) did not display additional bands. The purified protein bound [³²]IP₃ stoichiometrically, thus confirming its identity as the IP₃ receptor protein (data not shown).

In Vitro Phosphorylation of the IP₃ Receptor by cGMP Kinase and cAMP Kinase—The purified IP₃ receptor was phosphorylated in vitro by purified cGMP kinase and catalytic subunit of cAMP kinase. Autoradiography of the gel obtained on SDS-PAGE showed that both cGMP kinase and cAMP kinase catalyzed phosphorylation of the receptor within 5 min at 30°C (Fig. 2), suggesting that the IP₃ receptor compares favorably with the best substrates for cGMP kinase described to date.

Kinetics and Stoichiometry of Phosphorylation of the IP₃ Receptor—The time course of phosphorylation of the purified IP₃ receptor was examined. Both cAMP kinase and cGMP kinase-phosphorylated IP₃ receptor stoichiometrically with maximal phosphate incorporation observed at 5 min. 1 mol of phosphate was incorporated per mol of IP₃ receptor using 60 nM cAMP kinase, while 0.65 mol P/mol IP₃ receptor was incorporated using 30 nM cGMP kinase (Fig. 3). At a higher concentration of cGMP kinase (60 nM), the molar ratio increased to 0.89, whereas increasing cAMP kinase from 60 to 120 nM gave a molar ratio of 1.8, indicating that cGMP kinase is capable of catalyzing the phosphorylation of one site, while CAMP kinase can catalyze phosphorylation of two sites on the IP₃ receptor. Phosphorylation of IP₃ receptor was not additive using both cGMP and cAMP kinases in the phosphorylation assay (data not shown). It was possible that the lower extent of phosphorylation by cGMP kinase may be due to detergent interference in the sample, for which some kinases are more susceptible than others (29, 38).

Purification and Identification of Phosphopeptides—Phosphoamino acid analysis of the phosphorylated IP₃ receptor demonstrated that cGMP kinase and cAMP kinase-phosphorylated serine exclusively (data not shown). Two-dimensional mapping of the phosphorylated IP₃ receptor yielded one identical peptide whether cGMP kinase or cAMP kinase was used to catalyze phosphorylation of the protein (Fig. 4). However, the two-dimensional map obtained from the IP₃ receptor phosphorylated using the cAMP kinase displayed at least one additional spot.

In order to characterize the sites phosphorylated by the cGMP kinase and cAMP kinase, tryptic digests of the phosphorylated IP₃ receptor were separated by two or three steps of reverse-phase HPLC. Fig. 5 shows the HPLC chromatogram obtained from the first HPLC separation of phosphopeptides obtained from protein phosphorylated using the cGMP kinase or cAMP kinase. One identical radioactive peak that eluted at 13.8 min was obtained for the receptor phosphorylated using either cGMP kinase or cAMP kinase (Fig. 6). The minor peaks obtained were not studied further. The major radioactive peak eluted at 13.8 min in both cases was further purified on a second diphenyl HPLC column. One radioactive peak eluted at 14.1 min in both cases. This peak was rechromatographed on the same column to ensure purity (Fig. 7).
Characterization of the IP$_3$ Receptor Peptides (GRRESLTSFG and ARRDSVLAAAS)—Two-dimensional phosphopeptide mapping, HPLC analysis, amino acid composition, and partial sequence analysis of the phosphopeptides obtained from the IP$_3$ receptor phosphorylated using the cGMP kinase showed that the sequence of the phosphorylation site on the IP$_3$ receptor that is recognized by the enzyme was identical to the major site recognized by the cAMP kinase (serine-1755) (39). However, a minor phosphorylated site was previously identified for the cAMP kinase and was found to be serine-1589 (39). Because both cAMP and cGMP kinases have overlapping substrate specificities, we examined the phosphorylation of the synthetic peptides GRRESLTSFG and ARRDSVLAAAS corresponding to serine-1755 and -1589, respectively, from the IP$_3$ receptor. As shown in Figs. 8 and 9, GRRESLTSFG was phosphorylated rapidly and stoichiometrically by both kinases. Phosphorylation of this peptide was not additive when using both cGMP and cAMP kinases in the phosphorylation assay, and replacement of the second serine (1756) in the peptide with alanine did not affect the kinetics of phosphorylation (data not shown). The $K_m$ of the peptide for cGMP kinase was $35 \pm 3$ mM, while the $V_{max}$ was $6 \mu$mol/min/mg, making this the best known peptide substrate for cGMP kinase described to date. Phosphorylation of the peptide ARRDSVLAAAS corresponding to the minor site for the cAMP kinase (serine-1589) on the IP$_3$ receptor was examined. As shown in Fig. 10, this peptide was less effectively phosphorylated using cGMP kinase ($K_m$ for cGMP kinase = $66 \pm 2$ mM, and $V_{max}$ = 1.9 $\mu$mol/min/mg).

Phosphorylation of the IP$_3$ Receptor in Primary Culture of Rat Aortic Smooth Muscle Cells—In order to more clearly assess the potential importance for cGMP-catalyzed IP$_3$ receptor phosphorylation, the effect of cGMP on IP$_3$ receptor phosphorylation in intact cells was studied. Primary cultures of rat aortic smooth muscle prelabeled with $[^32P]orthophosphate and treated with atrial natriuretic peptide or a 0.1 mM atrial natriuretic peptide or 0.1 $\mu$M sodium nitroprusside resulted in elevated cGMP levels but not cAMP levels (data not shown). The IP$_3$ receptor was then extracted from the cells using procedures described for the purification of the protein and resolved by SDS-PAGE. As shown in Fig. 11, there was increased radioactivity in the IP$_3$ receptor band after treatment of cells with either 0.1 mM atrial natriuretic peptide or 0.1 $\mu$M sodium nitroprusside (approximately 30–40% compared with the control cells). Because the IP$_3$ receptor is a known substrate for other kinases such as protein kinase C and the Ca$^{2+}$/calmodulin-dependent protein kinase, there was a significant amount of radioactivity in the protein isolated from control cells. Nevertheless, these results demonstrate that elevation in cGMP levels may lead to the phosphorylation of the IP$_3$ receptor in intact vascular smooth muscle cells.

**DISCUSSION**

The results of this study are the first to show that the IP$_3$ receptor is phosphorylated using cGMP kinase. Phosphopeptide mapping and HPLC analysis of the phosphopeptides showed that cGMP kinase catalyzed the phosphorylation of one site on the IP$_3$ receptor. This was identical to the site phosphorylated by cAMP kinase. Ferris et al. (39) originally demonstrated that cAMP kinase catalyzed the phosphorylation of the IP$_3$ receptor in vitro on serine-1755. This site has the required RXS motif for cAMP kinase substrates and has, in addition, a phenylalanine residue four positions C-terminal to the phosphorylatable serine. Colbran et al. (40) have found that aromatic amino acids placed in this position increase selectivity for phosphorylation by cGMP kinase over cAMP kinase, although our results indicate that both cAMP and cGMP kinases catalyze phosphorylation of this site equally well. The high affinity (apparent $K_m$ = $35 \pm 3$ mM) and $V_{max}$ (6 $\mu$mol/min/mg) for the peptide corresponding to the phosphorylation site indicate that this is the best known substrate for cGMP kinase and compare favorably with the known physiological substrates for cGMP kinase, namely type V phosphodiesterase (41) and phospholamban (12). Because serine-1755 represents the major site phospho-
Phosphorylation of IP₃ Receptor by cGMP Kinase

FIG. 6. Radioactive profile of tryptic digests of IP₃ receptor phosphorylated by cGMP kinase (cGK) and cAMP kinase (cAK). The IP₃ receptor phosphorylated by either the cGMP kinase or cAMP kinase was subjected to trypsinization and purified by HPLC on a C8 column. The figure shows the identical profiles for the major peptide phosphorylated by either kinase.

FIG. 7. Rechromatography of the radioactive peak from the C8 column on a diphenyl column. The radioactive peak obtained from the C8 column was rechromatographed twice on a diphenyl column and eluted using a gradient of 0–70% acetonitrile. Absorbance was measured at 215 nm. A, radioactive peak obtained from cGMP kinase-phosphorylated IP₃ receptor; B, radioactive peak obtained from cAMP kinase-phosphorylated IP₃ receptor.

FIG. 8. Phosphorylation of IP₃ receptor peptide GRRESLTSFG by cGMP kinase (cGK) and cAMP kinase (cAK). Peptide (5–500 µM) was phosphorylated using cGMP kinase (21 nM) and catalytic subunit of cAMP kinase. Peptide was phosphorylated as described. This experiment has been repeated three times with 10% variation.

Phosphorylation of the IP₃ receptor could explain the results of Meisheri et al. (18) and Collins et al. (19) that elevations of cGMP inhibit IP₃ formation in some cells since Chinese hamster ovary cells transfected with the cDNA encoding cGMP kinase had reduced IP₃ levels in response to thrombin. Chinese hamster ovary cells, however, are not vascular cells and may not express vascular smooth muscle-specific proteins such as many of those present in sarcoplasmic reticulum. Nevertheless, the ability of cGMP kinase to inhibit both IP₃ receptor function as well as IP₃ generation could explain the greater capacity of cGMP to lower Ca²⁺ in agonist-stimulated (i.e. IP₃-mediated) cells, compared with depolarized cells (43, 44). We had reported earlier that cGMP kinase regulates Ca²⁺-ATPase activity in rat aortic smooth muscle cell sarcoplasmic reticulum by phosphorylation of phospholamban (12). When phosphorylated, phospholamban dissociates from ATPase, resulting in activation of the enzyme, leading to reduction of [Ca²⁺]. It is conceivable that the inhibition of Ca²⁺ release along with the stimulation of Ca²⁺ uptake in the sarcoplasmic reticulum may be one way rat aortic smooth muscle cells respond to cGMP in order to lower [Ca²⁺] and produce relaxation. The likelihood that it is cGMP kinase...
that catalyzes the phosphorylation of the IP3 receptor in rat aortic smooth muscle cells is supported by the fact that cGMP kinase, and not cAMP kinase, is localized to areas corresponding to the sarcoplasmic reticulum in rat aortic smooth muscle cells, and only cGMP kinase catalyzes phosphorylation of phospholamban in these cells (12). Since phospholamban and the IP3 receptor are both present in the sarcoplasmic reticulum, co-localization of the enzyme with these substrates may make them more accessible substrates for cGMP kinase versus cAMP kinase. Clearly, vascular smooth muscle relaxation produced by cGMP is a complicated event involving the phosphorylation of numerous proteins all involved in the reduction of [Ca2+]i or Ca2+ sensitivity in the intact cell. These include not only the IP3 receptor as described here, but also phospholamban, which regulates Ca2+-ATPase, unidentified proteins involved with the production of IP3, and unidentified proteins that regulate Ca2+.

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Phosphorylation of IP$_3$ Receptor by cGMP Kinase