Mechanisms in Bradykinin Stimulated Arachidonate Release and Synthesis of Prostaglandin and Platelet Activating Factor

Ricupero, D.

Hindawi Publishing Corporation

Ricupero, D., L. Taylor, A. Tlucko, J. Navarro, P. Polgar. "Mechanisms in bradykinin stimulated arachidonate release and synthesis of prostaglandin and platelet activating factor" Mediators of Inflammation 1(2): 133-140. (1982)

https://hdl.handle.net/2144/3221

Boston University
REGULATORY mechanisms in bradykinin (BK) activated release of arachidonate (ARA) and synthesis of prostaglandin (PG) and platelet activating factor (PAF) were studied in bovine pulmonary artery endothelial cells (BPAEC). A role for GTP binding protein (G-protein) in the binding of BK to the cells was determined. Guanosine 5'-O-(thiotriphosphate), (GTPS), lowered the binding affinity for BK and increased the Kd for the binding from 0.45 to 1.99 nM. The Bmax remained unaltered at 2.25 x 10^{-11} mole. Exposure of the cells to aluminium fluoride also reduced the affinity for BK. Bradykinin-induced release of ARA proved pertussis toxin (PTX) sensitive, with a maximum sensitivity at 10 ug/ml PTX. GTPS at 100 μM increased the release of arachidonate. The effect of GTPS and BK was additive at suboptimal doses of BK up to 0.5 nM but never exceeded the levels of maximal BK stimulation at 50 nM. PTX also inhibited the release of ARA induced by the calcium ionophore, A23187. Phorbol 12-myristate 13-acetate or more commonly known as tetracanoylphorbol acetate (TPA) itself had little effect on release by the intact cells. However, at 100 nM it augmented the BK activated release. This was down-regulated by overnight exposure to TPA and correlated with down-regulation of protein kinase C (PKC) activity. The down-regulation only affected the augmentation of ARA release by TPA but not the original BK activated release. TPA displayed a similar, but more potent amplification of PAF synthesis in response to both BK or the calcium ionophore A23187. These results taken together point to the participation of G-protein in the binding of BK to BPAEC and its activation of ARA release. Possibly two types of G-protein are involved, one associated with the receptor, the other activated by Ca^{2+} and perhaps associated with phospholipase A2 (PLA2). Our results further suggest that a separate route of activation, probably also PLA2 related, takes place through a PKC catalysed phosphorylation.

Key words: Arachidonate, Bradykinin, G-protein, Platelet activating factor, Prostaglandin synthesis

Introduction

The exposure of endothelial cells to bradykinin (BK), a nonapeptide with inflammatory as well as vasoactive functions, results in the activation of a number of early metabolic events as determined in a number of cell types. These events include a biphasic increase in cytosolic Ca^{2+}, a transient membrane hyperpolarization, the activation of K+ channels, formation of diacylglycerol (DAG) and phosphatidic acid, release of arachidonate (ARA) from phospholipid stores and synthesis of prostaglandin (PG) and platelet activating factor (PAF). Many of these events involve the activation of phospholipid related hydrolases including phospholipase A2 (PLA2) and phospholipase C (PLC).

Whereas the generation of inositol trisphosphate (IP3) and DAG are due to the activation of PLC, cumulative evidence suggests that the release of free arachidonic acid from phospholipid stores, at least in endothelial cells, involves PLA2 activation by BK. Although this release of fatty acid has been well described, the sequence of events which follows the binding of BK to the cell and results in the activation of PLA2 remains to be fully elucidated. The resulting increase in cytosolic Ca^{2+} is certainly a necessary step. However regulatory systems such as GTP-binding proteins (G-protein) and DAG activated protein kinase C (PKC) have
been implicated in the process.\textsuperscript{11,12} In this series of experiments we attempt to link some of these second messengers to elucidate the process of activation of PLA\textsubscript{2} by BK in the bovine pulmonary artery endothelial cell (BPAEC).

**Materials and Methods**

**Cell culture:** Endothelial cells were isolated without the use of proteolytic enzymes. A freshly obtained calf pulmonary artery was cut open and lightly scraped with a scalpel. The resulting clumps of endothelial cells were placed into 35 mm dishes containing McCoy's 5A [Sigma, St Louis, MO] medium supplemented with 20\% foetal bovine serum (FBS) [HyClone, UT].\textsuperscript{13} The homogeneity of endothelial cell cultures was determined morphologically and histochemically. At confluence the cells displayed a typical cobblestone appearance and stained positive for factor VIII antigen according to the method of Weinberg et al.\textsuperscript{14} Endothelial cells were maintained in 25 cm\textsuperscript{2} flasks containing 6 ml McCoy's 5A medium, 20\% FBS, 50 \mu g/ml streptomycin and 50 units/ml penicillin. The culture medium was replaced every 3 days and the cells passaged biweekly. Cells to be used for experiments were passaged from 25 cm\textsuperscript{2} flasks into 24-well plates, 20 cm\textsuperscript{2} dishes or 60 cm\textsuperscript{2} dishes at a density of 20 000 cells/cm\textsuperscript{2} with trypsin (0.05\%). The culture medium was replaced after 6 days. Two to 3 days after the last feeding, these cultures were used for the various experiments.

**Release of arachidonate:** Confluent cells in 24-well plates were prelabelled with \textsuperscript{3}H-arachidonate [79.9 Ci/mmol, New England Nuclear, MA] (0.2 \mu Ci per well) for 18 h. They were then washed once with McCoy's medium and preincubated for 2 h in McCoy's medium containing 1\% FBS (0.5 ml per well). This process was found previously to increase the potential of the cell to synthesize prostaglandin.\textsuperscript{13} In experiments with phorbol ester, 50 \mu l of a 10 \times concentration of TPA was added to the wells during the last 10 min of the preincubation. Cells were then incubated in McCoy's medium containing 2 mg/ml bovine serum albumin (BSA) (Sigma, St Louis, MO, essentially fatty acid free from essentially globulin free) and the indicated additions. After 10 min the medium was removed and centrifuged (800 \times g). Radioactivity was determined in a 200 \mu l aliquot of the supernatant.

**Radioimmunoassay (RIA) for prostaglandins:** Antibodies to 6-keto PGF\textsubscript{1\alpha} (6-K-PGF\textsubscript{1\alpha}) were prepared in our laboratory. PGF\textsubscript{1\alpha} concentrations were determined as its stable degradation product 6-K-PGF\textsubscript{1\alpha}. Cross-reactivity of the antisera against nontargeted PGs was less than 4\%.\textsuperscript{11} The radioimmunoassay was performed as described previously.\textsuperscript{13}

**Platelet activating factor:** The synthesis of PAF was determined as described previously.\textsuperscript{15} Generally the procedure of McIntyre et al. was followed.\textsuperscript{9} Confluent cells (20 cm\textsuperscript{2} Petri dishes) were incubated with \textsuperscript{3}H-acetate [3.6 Ci/mmol, New England Nuclear, Boston, MA] (50 \mu Ci/dish) in HEPES buffered Hank's balanced salt solution [Sigma, St Louis, MO], pH 7.4, with or without indicated additions. After 30 min at room temperature, the incubation was stopped by removing the medium and adding methanol/water (1:2) containing 50 mM acetic acid. The cells were scraped into this solution and transferred to screw-capped tubes containing chloroform. The plates were washed with methanol and the wash was added to the screw-capped tubes. After 2 h at room temperature, chloroform and water were added to split the monophase. The chloroform layer was dried under nitrogen and the lipids redissolved in small amounts of chloroform:methanol (2:1). The lipids were separated by thin layer chromatography (TLC) on alumina backed silica coated TLC plates in chloroform/methanol/acetic acid/water (25:14:4:2). Radioactivity was located by autoradiography and the radioactive spots were cut from the chromatogram and quantitated.

**Cell permeabilization:** In order that GTP\textsubscript{S} [Boehringer Mannheim, IN] could penetrate the BPAEC, the permeability of the cells was increased by a 3 min exposure to saponin (0.02 mg/ml) in McCoy's medium at room temperature. The saponin [Sigma, St Louis, MO] was removed and GTP\textsubscript{S} was added as indicated. This step was followed with vital stain.

**Binding of BK to cells:** The binding of BK to cells was carried out as described previously.\textsuperscript{16} Binding was done in 24-well plates at 4\textdegree C for 2 h. The cultures were washed three times with 1 ml of phosphate-buffered saline (72 mM NaCl, 1.6 mM KCl, 5 mM Na\textsubscript{2}HPO\textsubscript{4}, 0.9 mM KH\textsubscript{2}PO\textsubscript{4}) at 4\textdegree C. This was followed by a 15 min equilibration with 0.5 ml of modified Hank's Balanced Salt Solution (HBSS), pH 7.3, containing 0.05\% bovine serum albumin (BSA), 2 mM bacitracin, 10 mM HEPES, 120 mM N-methyl-D-glucamine (replacing NaCl), 0.65 mM Ca\textsubscript{Cl}\textsubscript{2}, 0.25 mM MgCl\textsubscript{2} and 0.25 mM MgSO\textsubscript{4}. This binding medium was removed and replaced with fresh, chilled binding medium containing the \textsuperscript{3}H-bradykinin [90 Ci/mmol, Amersham, IL]. Non-specific binding was determined in the presence of 3 \mu M unlabelled bradykinin. At the end of the incubation, the medium was aspirated and the cells were washed five times with 1 ml of the Modified Balanced Salt Solution with 0.2\% BSA. This was followed by two washes with 1 ml of
phosphate-buffered saline. Bound radioactivity was determined by solubilizing the cells with 0.5 ml of 0.2% sodium dodecyl sulphate. Radioactivity was quantitated in New England Nuclear [Boston, MA] 963 using an LKB Rackbeta Counter.

Protein kinase C: To determine PKC activity, cells were grown to confluence in 60 cm² dishes. They were treated with various effectors and then washed with cold PBS before harvesting by scraping into sample buffer (20 mM Tris buffer, pH 7.5 containing 0.33 M sucrose, 2 mM EDTA, 0.5 mM EGTA, and 100 µg/ml leupeptin). The scraped cells were centrifuged at 2 000 × g and the PKC was solubilized in 1% Triton X 100 in sample buffer for 30 min on ice. The insoluble material was removed by centrifugation and the supernatant containing the PKC was absorbed to DE52 resin. The resin was then eluted from the resin with sample buffer containing 100 mM NaCl. Activity was determined as described by Navarro et al. Aliquots of the enzyme were mixed with 10 mM MgCl₂, 100 µM T²³P ATP (1 0000 dpm/pmol), 50 µg of histone III-S with or without 1 mM CaCl₂, 5 µg of phosphatidylserine, and 20 ng of phorbol dibutyrate (PDBu) in a final volume of 50 µl. Samples were incubated for 10 min at 30°C. The reaction was stopped by spotting 25 µl of the reaction mixture onto Whatman 3MM paper and then washing the filter papers in 10% trichloroacetic acid containing 10 mM sodium pyrophosphate. Protein kinase C activity was determined by subtracting the amount of incorporation into histone in the absence of added Ca²⁺, phosphatidylserine, and PDBu.

Assay for sn-1,2-diacylglycerol: Diacylglycerol was determined according to the method of Preiss et al. Cells were grown in 20 cm² plates and treated as indicated in the figure legends. Lipids were extracted and the dried lipids solubilized in 20 µl of an octyl-B-D-glycoside/cardiolipin solution (7.5% octyl-B-D-glycoside, 5 mM cardiolipin in 1 mM diethylenetriaminepentaacetic acid (DETAPAC) by sonication in a bath sonicator). Fifty microlitres reaction buffer (100 mM imidazole HCl, pH 6.6, 100 mM NaCl, 25 mM MgCl₂ and 2 mM EGTA), 2 µl 100 mM dithiothreitol, 10 µl diluted membranes containing DAG kinase (5 µg protein) and 8 µl water were then added. The reaction was started with the addition of 10 µl 10 mM (γ²³P) ATP prepared in 100 mM imidazole, 1 mM DETAPAC, pH 6.6. After 30 min at 25°C the reaction was stopped with chloroform/methanol and the lipids extracted. The chloroform layer was washed twice with 2 ml 1% HClO₄. The volume of the chloroform layer was measured and an aliquot removed and dried under nitrogen for TLC. Silica Gel 60 thin layer chromatography plates were activated by running in acetone and air dried immediately before spotting samples. Plates were developed with chloroform:methanol:acetic acid (65:15:5), air dried and spots located by autoradiography. Radioactivity was quantitated by counting the scraped silica in a scintillation counter. The amount of 1,2-DAG present in the original sample was calculated from the sample volumes and the specific activity of the ATP.

Results

The effect of GTPγS on binding of BK to BPAEC: To determine interaction between bradykinin, G-protein and phorbol 12-myristate 13-acetate (TPA) we first looked at the effect of GTPγS on the binding of BK to endothelial cells. This was done in intact cells which were permeabilized with saponin. A typical binding of BK to endothelial cells is illustrated as a binding curve (inset) and its Scatchard plot in Fig. 1. The permeabilization itself had no effect on the binding. The untreated cells bound with a Kd of 0.45 nM. Adding GTPγS (100 µM) to the incubations solution had a marked negative effect on the binding, increasing the Kd to 1.99 nM. Bmax stayed constant as 2.2 × 10⁻¹¹ mole. GDPβS, structurally similar to GTPγS but not an activator of G-protein, had no effect on binding (data not shown). In a separate experiment, aluminium fluoride, which dissociates the G-protein, reduced the binding of BK significantly as illustrated in Fig. 2. ATP (10 µM) had no effect.

The effect of GTPγS on release of ARA: As illustrated in Fig. 3, the release of arachidonate from endothelial cells preincubated with ³H-ARA is related to the GTPγS concentration up to approximately 100 µM. This release is time dependent as illustrated in Fig. 4. In this figure the release of arachidonate by GTPγS (100 µM) is compared to that by BK (50 nM). At 2 min after addition, BK more than doubles the release of label while GTPγS has little effect. Some effect by GTPγS is seen at 5 and 10 min, but this is small in comparison to BK. At 20 min after stimulation the effect by GTPγS is 70% that of BK. The effect of pertussis toxin (PTX) was tested with regard to the release of arachidonate and PG synthesis. As illustrated in Fig. 5, PTX had a negligible inhibition of release by cells not treated with BK. The release caused by BK (50 nM) was approximately 12-fold the basal value. PTX reduced the BK activated increase of arachidonate release to approximately twice that of the basal value. Interestingly, PTX also blocked the release caused...
FIG. 1. The binding of BK to BPAEC: effect of GTP·S. Confluent cells were washed and incubated in medium containing 1% FBS for 2 h. They were then permeabilized with McCoys’s medium with saponin (0.02 mg/ml) for 3 min. The cells were incubated in fresh medium with or without GTP·S (100 µM) for 10 min. After this incubation, the cells were rapidly chilled by placing on ice and washing with ice cold phosphate buffered saline. Binding was performed as described in the methods section. Scatchard plot was generated using the Ligand program. O = specific binding (total binding—nonspecific); • = specific binding in presence of GTP·S.

by A23187 to a similar degree. The effect was related to the PTX concentration as illustrated in Fig. 6. Some effect is seen at 1 ng/ml; by 10 ng/ml maximum effect is seen. No further inhibition is detected at 100 ng/ml.

The interaction between BK and GTP·S on the release of arachidonate is illustrated in Fig. 7. GTP·S alone at 100 µM increased release. BK increased release from 0.5 nM up to 5 nM. At suboptimal concentrations of BK, the addition of GTP·S increased release above that of BK up to 0.5 nM BK. Above this concentration of BK GTP·S had no additive effect.

Phorbol 12-myristate 13-acetate: Exposure of BPAEC to TPA (100 nM) for 10 min in absence of BK had no effect on the release of arachidonate (Fig. 8). However, as illustrated in the same figure, TPA increased BK stimulated release. When the cells were treated overnight with TPA (500 nM) to down-regulate PKC activity and then were pretreated with fresh TPA and exposed to BK the

FIG. 2. Effect of aluminum fluoride on BK binding to BPAEC. Confluent cells were washed and incubated in medium containing 1% FBS for 2 h. They were then placed on ice and washed with ice-cold phosphate buffered saline. Sodium fluoride (25 mM) and aluminium chloride (10 µM) were added in cold binding buffer containing 3H-bradykinin (0.5 nM) plus or minus unlabelled bradykinin (3 µM). Binding was determined as described in the methods section.

FIG. 3. Concentration related effect of GTP·S on the release of ARA. Confluent cells were labelled with 3H-arachidonate for 18 h. Cells were then washed and incubated in medium containing 1% FBS for 2 h. Cells were permeabilized by incubation with McCoys’s medium plus saponin (0.02 mg/ml) for 3 min. The cells were washed and incubated in medium containing 2 mg/ml BSA and the indicated concentrations of GTP·S. After 15 min medium was removed and arachidonate release determined as described in the methods section. Results are the mean of quadruplicate cultures.

FIG. 4. Time course of BK and GTP·S effect on release of ARA. Cells were treated as described in Fig. 3 except that medium was harvested at the indicated times after bradykinin (50 nM) or GTP·S (100 µM) addition. □ = BK treated ○ = GTP·S treated △ = Non-stimulated release
FIG. 5. The effect of PTX on BK and A23187 stimulated release of ARA. Confluent cells were labelled with ³H-arachidonate for 18 h. The cells were washed and incubated in medium containing 1% FBS with or without pertussis toxin (10 ng/ml). After 4 h the medium was removed and medium containing BSA (2 mg/ml) with or without BK (50 nM) or A23187 (10 μM) was added. After 15 min, medium was harvested from quadruplicate wells and radioactivity determined. Results are the means of quadruplicate cultures.

Cells not treated with PTX

Cells treated with PTX

response to BK remained unaltered, but no augmentation by TPA was observed (Fig. 8). Determination of PKC activity, as illustrated in Fig. 8 (insert) shows that PKC was indeed downregulated by exposure of the cells to 500 nM TPA overnight. In Fig. 9 a similar effect by TPA is seen in BK activated PAF synthesis. In this case TPA at 16 nM increased BK-activated PAF production by approximately three-fold. Interestingly the response to A23187, a calcium ionophore, was also augmented by TPA.

As a further illustration of the participation of PKC in BK activated release, Fig. 10 shows that in response to BK, BPAEC produce DAG. DAG

FIG. 6. Inhibitory effect of PTX at various concentrations. Confluence cells were washed and incubated in medium containing 1% FBS and the indicated concentration of pertussis toxin (PTX). After 4 h this medium was removed and the cells incubated in McCoy's medium with or without bradykinin (50 nM) or ionophore A23187 (10 μM). After 20 min medium was harvested from quadruplicate wells and assayed for 6-keto-PGF₁α content by RIA.

control

+ BK

+A23187

FIG. 7. Synergism between BK and GTPS in the release of ARA. Cells were treated as described in Fig. 3 except that they were incubated with the indicated concentrations of BK plus or minus GTPS (100 μM).

Cells not treated with PTX

Cells treated with PTX

FIG. 8. The effect of down-regulation on ARA release by TPA in BK treated cultures, concomitant down-regulation of PKC. Confluent cultures were labelled with ³H-arachidonate for 18 h. For PKC down-regulation TPA (500 nm) was included during this 18 h incubation. The cells were washed and the medium changed to medium containing 1% FBS (0.5 ml/well). After 2 h 50 μl of a 1 μM solution of TPA was dropped into the appropriate wells. After 10 min the medium was removed and medium containing 2 mg/ml BSA with or without BK (50 nM) was added. After 10 min this medium was collected and arachidonate release determined as described in the methods section. Results are the means of quadruplicate cultures. Insert: Parallel cultures were set up in 60 cm² Petri dishes. They were treated with the indicated concentrations of TPA for 18 h. PKC activity was determined as described in the Methods section.

Cultures not treated with TPA

Cultures treated with 100 nM TPA

Mediators of Inflammation· Vol 1 · 1992  137
production was determined at various concentrations of BK from 0.01 to 50 nM. In this case a maximum was reached between 0.5 and 2 nM BK.

The interaction of TPA with BK and GTP\(\gamma\)S is illustrated in Fig. 11. The release of arachidonate was determined with either BK or GTP\(\gamma\)S in the presence or absence of TPA. In this case TPA basically doubled the effect of BK. It proved to have no effect on GTP\(\gamma\)S stimulated release.

**Discussion**

In the experiments described we investigated the high affinity B\(_2\) binding site of BK in intact, viable endothelial cells. We found evidence in these cells of only one high affinity B\(_2\) site.\(^{16}\) Results illustrated in Figs 1 and 2 show that this binding of BK involves G-protein. The addition of GTP\(\gamma\)S, a non-hydrolysable analogue of GTP, to cells previously exposed to saponin increased the Kd of binding by approximately four-fold (0.45 nM to 1.99 nM) while the B\(_{\text{max}}\) remained unchanged at 2.25 \(\times\) 10\(^{-11}\) mole. This suggests dissociation of G-protein leading to a lower affinity for BK. GTP\(\gamma\)S is known to maintain the G-protein in a permanently dissociated state. Exposure of the cells to aluminium fluoride, which dissociates G-protein, also lowered the binding of BK to the BPAEC. These experiments, conducted at the cellular level with the receptor intact, are consistent with reports using plasma membranes isolated from fractionated myometrium\(^{9,20}\) and the bovine aorta.\(^{21}\) Our results are also consistent with the recent report of the cloning of the B\(_2\) receptor for BK from rat uterus as a typical G-protein coupled receptor.\(^{22}\)

Separate G-proteins appear to function in the regulation of PLA\(_2\) and PLC. A number of reports suggest that the generation of inositol triphosphate (IP\(_3\)) is not PTX sensitive. In contrast, the action of PLA\(_2\) is PTX sensitive. For example, in 3T3 fibroblasts, thrombin and BK increase Ca\(^{2+}\) influx, arachidonate release and IP\(_3\) release. PTX was reported to inhibit arachidonate release but not IP\(_3\) release.\(^{23}\) In FRTL5 thyroid cells, adrenergic \(\alpha\) receptors are coupled to PLA\(_2\) by a PTX sensitive G-protein, and to PLC by a PTX insensitive G-protein.\(^{24}\)
PTX-insensitive G-protein is associated directly with the BK receptor while the PTX-sensitive G-protein may be associated directly with PLA₂. The G-protein associated with the receptor may be involved in the PLA₂ regulation of cytosolic Ca²⁺. Clark et al. have demonstrated that endothelial cells express mRNA for all three subtypes of Gi.

Our results illustrate that TPA is also involved in the release of arachidonate in BPAEC. Generally phorbol ester has been shown to inhibit the action of PLC in the generation of IP₃. This inhibition is related to PKC. With regard to the activation of PLA₂ by phorbol ester, the literature is more tenuous. For example, in the experiments using MDCK cells, TPA itself stimulated the release of arachidonic acid which was inhibited by 1-(5-isquinolinsulphonyl)-2-methylpiperazine (H7), an inhibitor of PKC. In another report using MDCK cells, the BK response was only slightly sensitive to PKC inhibitors (spingosine, H7, staurosporine). The BK-stimulated release of arachidonic acid could not be enhanced by TPA in PKC-down-regulated BPAEC. However, in these cells TPA itself stimulated the release of arachidonic acid which was attenuated (50%) by PKC inhibitors. Phorbol ester was reported to augment BK-stimulated PG synthesis in other cell types such as Swiss 3T3. Other effectors such as thrombin, which activates PG and PAF synthesis in human endothelial cells, are augmented by TPA. In the described experiments, TPA alone did little in the absence of BK. However, in the presence of BK the release of arachidonate was synergistic. This synergism by phorbol was abolished when PKC was down-regulated by incubating the cells overnight with TPA. However, the BK-stimulated release was totally unaffected by the down-regulation of PKC. This action by TPA appears related to PLA₂. This is suggested in Fig. 9 by the TPA-augmented, BK-induced synthesis of PAF. In these experiments the synthesis of PAF was determined through the remodelling pathway which utilizes PLA₂ to remove fatty acid from the sn-2 position of 1-O-alkyl-2-acetyl-sn-glycelyl-3-phosphocholine and incorporates acetate into the lyso-PAF via lyso-PAF acetyl CoA acetyltransferase (LPAT) to form the 1-O-alkyl-2-acetyl-sn-glycelyl-3-phosphocholine (PAF). It is possible that TPA in conjunction with BK also activates PLA₂. This is suggested by the considerably larger augmentation by TPA of BK-stimulated PAF synthesis than of ARA release. In fact Heller et al. reported recently that thrombin-activated PAF synthesis in human umbilical vein endothelial cells involved the activation of PLA₂. TPA alone did not activate PLA₂. The effect of thrombin plus TPA on PLA₂ activity was not determined. TPA also did not affect GTPγS activation of release (Fig. 11), suggesting that the two events represent separate paths to the activation of PLA₂. As we illustrated, BK does generate DAG (Fig. 10), and thus may, under certain conditions, additionally activate the release of arachidonate through the action of protein kinase C.

References

1. Colden-Stanfield M, Schilling WP, Ritchie AK, Eskin SG, Navarro LT, Kunze DL. Caé Res 1987; 41:632-640.
2. Schilling WP, Ritchie AK, Navarro LT, Eskin SG. Am J Physiol 1988; 255:H1215-H1227.
3. Jones A, Langan TW, Lodge NJ, Ryan US, Van Breeemen C, Adams DJ. Tissue Cell 1987; 19:733-745.
4. Buchan KW, Martin W. Br J Pharmacol 1991; 102:35-40.
5. Mohrke PU, Ducu J. J Physiol (Lond) 1991; 439:271-299.
6. Thuringer D, Diarra A, Sause R. Am J Physiol 1991; 261:H1656-H1666.
7. Graier WP, Schmidt K, Kurvers MR. Cell Signal 1990; 2:369-375.
8. Hong SL, Deskyin D. J Biol Chem 1982; 257:7115-7154.
9. McIntyre TM, Zimmerman GA, Starch KH, Prescott SM. J Clin Invest 1987; 78:271-280.
10. Kays H, Parrott GM, Hong SL. J Biol Chem 1989; 264:4972-4977.
11. Burch RM. FEBS Lett 1989; 234:283-286.
12. Burch RM. Mol Endocrinol 1989; 3:155-171.
13. Menconi M, Hahn G, Polgar P. J Cell Physiol 1984; 120:163-168.
14. Wieland KS, Douglas WHJ, McNamara DR, Lanzillo JJ, Fanburg BL. In Vivo 1985; 18:400-406.
15. Heinson C, Polgar P, Fishman J, Taylor L. Arch Biochem Biophys 1987; 287:251-258.
16. Cahill M, Fishman JB, Polgar P. Agents and Actions 1990; 24:224-229.
17. Navarro J. J Biol Chem 1987; 262:4649-4652.
18. Preiss J, Loomis CR, Bishop WR, Stein RI, Niedel J. Bell RM. J Biol Chem 1987; 262:4649-4652.
19. Liebman C, Offermanns S, Hirsch KD, Schneider M, Moravy JI, Reissmann S, Schulte G, Rosenhe W. Biochem Biophys Res Comm 1990; 167:910-917.
20. Leech-Lundberg LM, Mathis SA. J Biol Chem 1990; 265:9621-9627.
21. Kunze DL. Circ Res 1987; 61:632-640.
22. Goldstein SA, Richter AK, Eskin SG, Navarro LT, Kunze DL. Caé Res 1987; 41:632-640.
23. Cleemann W, Rutten GM, DeLacroix MF, Regoli D, Hiley CR, Stoclet JC. Tissue Cell 1987; 19:733-745.
D. Ricupero et al.

24. Burch RM, Luini A, Axelrod J. Proc Natl Acad Sci USA 1986; 83: 7201–7206.
25. Voyno-Yasenetskaya TA, Tkachuk VA, Chkhenyova EG, Panchenko MP, Grigorian GY, Vavrek RJ, Stewart JM, Ryan US. Faseb J 1989; 3: 44.
26. Nakahima S, Nogita K, Ueda K, Nozawa Y. Arch Biochem Biophys 1988; 261: 373–383.
27. Bochkov VN, Feoktistov IA, Avdotin PV, Tkachuk VA. Biochemistry 1989; 28: 1533–1542.
28. Weiss C, Atlas D. Brain Res 1991; 543: 102–110.
29. Clark MA, Conway TM, Bennett CF, Crook ST, Sudel JM. Proc Natl Acad Sci USA 1986; 83: 7320–7324.
30. Lee RT, Brock TA, Tolman CJ, Siedman JG, Neer E. FEBS Lett 1989; 249: 139–142.
31. Portilla D, Mordhorst M, Bertrand W, Morrison AR. Biochem Biophys Res Comm 1988; 158: 454–459.
32. Weiss BA. Am J Pharm and Exp Ther 1989; 36: 317–326.
33. Burch RM, Lin MA, Axelrod J. J Biol Chem 1988; 263: 4764–4767.
34. Zavoico GB, Hebolich JK, Gimbrone MA Jr., Schafer Al. J Cell Physiol 1990; 143: 596–605.
35. Heller R, Bussolino F, Ghigo D, Garbarino G, Pescarmona G, Till U, Bossa A. J Biol Chem 1991; 266: 21358–21361.

ACKNOWLEDGEMENTS. This work was supported by NIH Grants HL25776 and AG00115.

Received 6 February 1992; accepted in revised form 25 February 1992