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MULTIPLE MECHANISMS OF ARACHIDONIC ACID RELEASE IN CHINESE HAMSTER OVARY CELLS TRANSFECTED WITH cDNA OF SUBSTANCE P RECEPTOR

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Abstract—We investigated the release of [³H]arachidonic acid ([³H]AA) and its relationship to the formation of [³H]inositol trisphosphate ([³H]IP₃) elicited by substance P (SP) in prelabeled Chinese hamster ovary cells stably expressing the SP receptor. Activation of the SP receptor resulted in a concentration- and time-dependent stimulation of [³H]AA release. Half-maximal release was obtained at 10^{-9} M, comparable to that for [³H]IP₃ formation reported previously, and the maximal release effected by 0.1 μM SP was 8 to 10-fold above the basal value. Both the [³H]AA release and the [³H]-IP₃ accumulation stimulated in the cells by 0.1 μM SP were concentration-dependently blocked with the specific SP receptor antagonist CP-96,345, with IC_{50} values of 2.5 and 0.4 μM, respectively. The time course of [³H]AA release showed a biphasic pattern: an initial rapid release essentially independent of Ca^{2+}, followed by a sustained release markedly suppressed by removal of extracellular Ca^{2+} or chelation of intracellular Ca^{2+} with 1,2-bis(2-aminophenoxyethane)-N,N,N',N'-tetraacetic acid (BAPTA). While pretreatment with pertussis toxin (200 ng/mL, 6 hr) did not block [³H]IP₃ formation, it did reduce [³H]AA release by 50% at 1 and 10 min after SP stimulation. Treatment of the cells with a phorbol ester, a protein kinase C activator, augmented the SP-stimulated [³H]AA release, and sphingosine, a protein kinase C inhibitor, reversed the phorbol ester-potentiated [³H]AA release, but not the release stimulated by SP alone, suggesting a synergistic effect of protein kinase C on SP-stimulated AA release. These results demonstrate that SP, acting at the SP receptor, stimulates [³H]-AA release via mechanisms that are (1) mediated by a pertussis toxin-sensitive G-protein, (2) dependent on extracellular Ca^{2+}, and (3) enhanced by activation of protein kinase C.

Key words: arachidonic acid; inositol trisphosphate; substance P; CHO cell

SP‖ is a widely distributed neuropeptide of the tachykinin family and has been implicated in motor and sensory, cardiovascular, and gastrointestinal functions [1–3], as well as in immunological and inflammatory responses [4, 5]. SP receptor belongs to the family of G-protein-coupled receptors with seven transmembrane segments and mediates the stimulation of PI hydrolysis through activation of PLC in many tissue preparations and different cell types [6–8]. An increase in $[Ca^{2+}]_i$ induced by this peptide also has been reported [9, 10]. Receptor-mediated mobilization of AA from phospholipids is of particular importance because AA serves as the rate-limiting precursor for the production of many biologically active metabolites through at least three pathways: cyclooxygenases, lipoxygenases, and P450 epoxygenases [11, 12]. AA and its metabolites, including prostaglandins and leukotrienes, act as first and second messengers to modulate a number of cellular processes [11–14]. In fact, liberation of prostaglandins in response to SP is considered to be relevant to the effects of SP on inflammation, vasoconstriction, and nociception [4, 15–17].

There are at least two pathways that can give rise to the receptor-mediated release of AA. Receptors may release AA indirectly by stimulating PI hydrolysis, which generates diacylglycerol along with IP_3. Diacylglycerol activates protein kinase C, and the concomitant formation of IP_3 releases Ca^{2+} from intracellular stores and increases $[Ca^{2+}]_i$. A recent study with CHO cells expressing cytosolic PLA_2 has clearly demonstrated the importance of both a rise in $[Ca^{2+}]_i$ and phosphorylation of PLA_2 as synergistic mechanisms for regulating the hormonal activation of PLA_2 [18]. Diacylglycerol may be further metabolized by diacylglycerol lipase to produce AA [19]. On the other hand, accumulating

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‖ Abbreviations: SP, substance P; PI, phosphatidylinositol; PLC, phospholipase C; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; AA, arachidonic acid; IP_3, inositol trisphosphate; CHO, Chinese hamster ovary; PLA_2, phospholipase A_2; CHO-SPR, Chinese hamster ovary cells transfected with cDNA for the SP receptor; α-MEM, α-minimal essential medium lacking ribonucleosides and deoxyribonucleosides; TPA, 12-O-tetradecanoylphorbol 13-acetate; 4α-PDD, 4α-phorbol 12,13-didecanoate; and BAPTA/AM, acetomethoxy esters of 1,2-bis(2-aminophenoxyethane)-N,N,N',N'-tetraacetic acid.

evidence from several laboratories has suggested that receptors may activate PLA$_2$ via a G-protein-coupled mechanism that is parallel to and independent of PI metabolism, which then directly hydrolyzes membrane phospholipids to release AA [20, 21]. Although SP was reported previously to produce both PI hydrolysis and prostaglandin release in astrocytes cultured from rat neonatal spinal cord [17, 22], it was not clear from these reports whether the SP-evoked formation of the AA metabolites prostaglandins D$_2$ and E$_2$ was linked to PI metabolism and/or a G-protein. Because of the existence of multiple tachykinin receptors [8], the precise characterization of signal transduction underlying SP receptors has been limited in cells in primary culture [22]. The transfection and functional expression of the cDNA clone for individual tachykinin receptor in cultured cells provide a model system with which to study precise signal transduction of a single receptor without any ambiguity [23–25]. In CHO-SPR cells, we concluded that the SP receptor is coupled to the stimulatory cascades of both PI hydrolysis and cyclic AMP formation [25]. In the present study, we further demonstrate that SP stimulated [³H]AA release from prelabeled CHO-SPR cells. The SP-stimulated release was mediated through both Ca^{2+} mobilization resulting from PI metabolism and a pertussis toxin-sensitive G-protein.

MATERIALS AND METHODS

Materials. The SP receptor antagonist CP-96,345 was a gift from Pfizer Inc. (Groton, CT, U.S.A.). Commercial sources of materials used were as follows: [5,6,8,9,11,12,14,15-³H]AA (60 Ci/mmol), Du Pont-New England Nuclear (Boston, MA, U.S.A.); *myo*-[2-³H]inositol (20 Ci/mmol), Amersham (Arlington Heights, IL, U.S.A.); SP, Peptide Institute (Osaka, Japan); α-MEM and Hanks' balanced salt solution, GIBCO (Grand Island, NY, U.S.A.); fetal bovine serum, Summit Biotechnology (Cleveland, OH, U.S.A.); TPA, 4α-PDD, and pertussis toxin, Funakoshi Pharmaceuticals (Tokyo, Japan); and BAPTA/AM, Dojindo Laboratories (Kumamoto, Japan). All other chemicals were of reagent grade. CHO-SPR cells stably expressing the SP receptor were maintained in α-MEM with 10% fetal bovine serum as described previously [25].

Measurement of [³H]AA release. Subconfluent cultures of CHO-SPR cells in 6-well plates (5 × 10^5 cells/well) were labeled by incubation with 0.5 μCi/well of [³H]AA in α-MEM containing 5% fetal bovine serum in a humidified atmosphere at 37° for 24 hr. Unincorporated label was removed by washing twice with α-MEM supplemented with 0.2% BSA and 20 mM HEPES, pH 7.4. Then the cell cultures were incubated in 2 mL of the same medium with or without the agonist. After the indicated times, the incubation media were collected and centrifuged to remove nonadherent cells. Duplicate aliquots were examined for total radioactivity in a Packard Tri-Carb 2200 CA liquid scintillation analyzer. In assays for the effect of Ca^{2+} on [³H]AA release, Hanks' balanced salt solution supplemented with 1.4 mM CaCl$_2$ and 0.6 mM MgCl$_2$, instead of the α-MEM mentioned above, was used as Ca^{2+}-containing

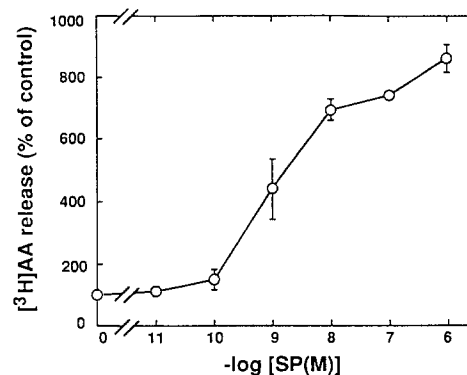Fig. 1. Concentration–response curve of SP for stimulation of [³H]AA release from CHO-SPR cells. Confluent monolayers were prelabeled with [³H]AA (0.5 μCi/well) for 24 hr. For each assay, the cells were washed twice with α-MEM containing 20 mM HEPES and 0.2% BSA, and release of label into the same medium elicited by various concentrations of SP was determined over a 10 min period at 37°. Data are expressed as a percentage of the basal release (710 ± 90 dpm/well). Each point represents the mean ± SEM (N = 6).

medium. Ca^{2+}-free medium comprised Hanks' balanced salt solution supplemented with 0.6 mM MgCl$_2$ and 1 mM EGTA. The data reported were obtained from at least two separate experiments done in triplicate.

Measurement of [³H]IP$_3$ accumulation. CHO-SPR cells cultured in 12-well plates (2 × 10^5 cells/well) were labeled with *myo*-[³H]inositol (1 μCi/well) in inositol-free Dulbecco's modified Eagle's medium supplemented with 10% dialyzed fetal bovine serum for 24 hr. The cells were then washed twice with HEPES-buffered saline solution containing 125 mM NaCl, 4.7 mM KCl, 1.2 mM KH$_2$PO$_4$, 2.2 mM CaCl$_2$, 1.2 mM MgCl$_2$, 15 mM NaHCO$_3$, 11 mM glucose, and 15 mM HEPES, pH 7.4, and preincubated in HEPES-buffered saline solution supplemented with 10 mM LiCl for 15 min at 37°. Reactions were started by the addition of test agents. For termination of the reaction, the medium was aspirated quickly, and 5% (w/v) trichloroacetic acid solution was added to each well. Separation of [³H]inositol phosphates was carried out by Bio-Rad AG1 × 8 chromatography essentially as described previously [25]. Radioactivity in the eluates was determined by scintillation counting using Clearsol (Nacalai Tesque, Kyoto, Japan).

The time course of [³H]AA release by 0.1 μM SP in HEPES-buffered saline solution supplemented with 10 mM LiCl was almost the same as that in the medium for the [³H]AA release study (data not shown).

RESULTS

Concentration dependence of SP for [³H]AA release from CHO-SPR cells. SP stimulation of CHO-SPR cells prelabeled with [³H]AA caused a concentration-dependent increase in radioactivity
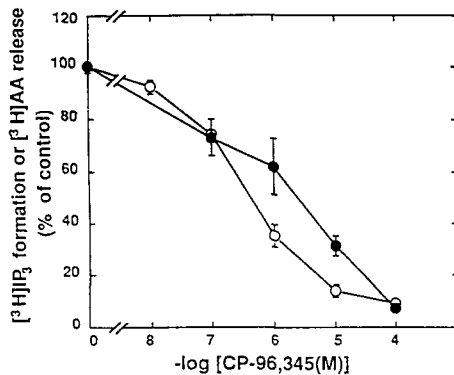
Fig. 2. Concentration-dependent inhibition by CP-96,345 of SP-stimulated [³H]AA release and [³H]IP₃ formation. Confluent monolayers were prelabeled with [³H]AA (0.5 μCi/well) or myo-[³H]inositol (1 μCi/mL) for 24 hr. CP-96,345 was added 15 min before the addition of 0.1 μM SP. [³H]AA release (●) and [³H]IP₃ formation (○) for 10 min were measured as described under Materials and Methods. Data are means ± SEM of experiments performed in triplicate and expressed as a percentage of the value in the absence of CP-96,345 (3460 ± 530 dpm/well for [³H]-AA release or 4210 ± 300 dpm/well for [³H]IP₃ formation).

Fig. 3. Time courses of [³H]AA release and [³H]IP₃ formation in CHO-SPR cells affected by SP. Confluent monolayers were prelabeled with [³H]AA or myo-[³H]-inositol as described in the legend for Fig. 2. (A) shows the release of [³H]AA after stimulation with 0.1 μM SP for the indicated times at 37°. The inset indicates the time course of [³H]AA release caused by 1 μM SP during a 120-sec incubation period. (B) shows [³H]IP₃ formation after stimulation with 1 μM SP. The same time course of [³H]-IP₃ formation was obtained with 0.1 μM SP. Values are expressed as a percentage of the basal level and presented as means ± SEM (N = 3). The dpm/well of the basal level was 550 ± 60 for [³H]AA release and 470 ± 30 for [³H]IP₃ formation.

release into the medium. As shown in Fig. 1, SP was a potent agonist for [³H]AA release; the release was observed at a concentration as low as 10^{-10} M with an EC_{50} value of 10^{-9} M, comparable to that for [³H]IP₃ formation in CHO-SPR cells reported previously [25]. No agonist-induced [³H]AA release was observed in untransfected CHO cells (data not shown).

Effects of SP receptor antagonist CP-96,345 on SP-stimulated [³H]AA release and [³H]IP₃ formation. Cultured CHO-SPR cells were preincubated for 15 min with different concentrations of the potent and selective SP receptor antagonist CP-96,345 [26]. This antagonist blocked both [³H]AA release and [³H]IP₃ accumulation in cells stimulated with SP (0.1 μM) in a concentration-dependent manner with IC_{50} values of 2.5 and 0.4 μM, respectively (Fig. 2). These results confirmed that stimulation of both responses is mediated by activation of the SP receptor.

Time courses of SP-stimulated [³H]AA release and [³H]IP₃ formation. To determine whether PI hydrolysis was a prerequisite for the SP-mediated [³H]AA release, we compared the time courses of both responses. SP-stimulated [³H]AA release was rapid and detected at 15 sec, as early as [³H]IP₃ formation, and more than a 10-fold increase in the release over the basal level occurred by 90 sec (see inset of Fig. 3A). The release gradually increased over an incubation period of 10 min after the rapid release that was seen within 2 min (Fig. 3A). Consistent with our previous results [25], when myo-[³H]inositol-labeled CHO-SPR cells were exposed to 1 μM SP, [³H]IP₃ levels increased for 10 min following the initial peak detected within 1 min (Fig. 3B). Both products reached a level 11-fold above the basal level by 10 min after SP challenge. Similar time courses of [³H]AA release and [³H]IP₃ formation
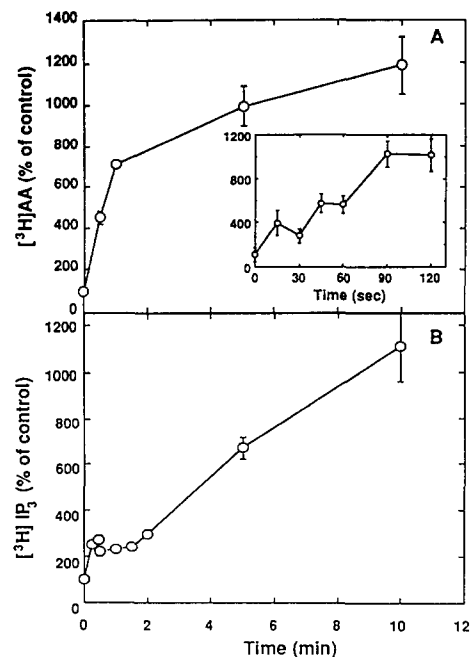
most likely suggest a simultaneous elicitation of both responses by SP receptor activation rather than one response preceding the other.

Role of extracellular and intracellular Ca^{2+} in the SP-stimulated [³H]AA release and [³H]IP₃ accumulation. Next we examined the effects of Ca^{2+} on [³H]AA release and [³H]IP₃ accumulation. As shown in Fig. 4A, the exposure of CHO-SPR cells to a Ca^{2+}-free medium in the presence of 1 mM EGTA for 15 min did not suppress the SP-stimulated [³H]AA release at 1 min after stimulation. On the other hand, SP-stimulated release was reduced by 54.1 and 62.6% at 5 and 10 min, respectively, when compared with the release in the Ca^{2+}-containing medium. To further examine the dependency on Ca^{2+} of [³H]AA release stimulated by SP receptor activation, we chelated the intracellular Ca^{2+} by treatment of the cells with the membrane-permeant, non-chelating compound BAPTA/AM. Intracellular hydrolysis of BAPTA/AM leads to the progressive accumulation of the Ca^{2+} chelator BAPTA within the cytosol, thus lowering $[Ca^{2+}]_i$ [27]. To ensure that intracellular Ca^{2+} stores were indeed depleted by BAPTA loading, 1 mM EGTA was added to the
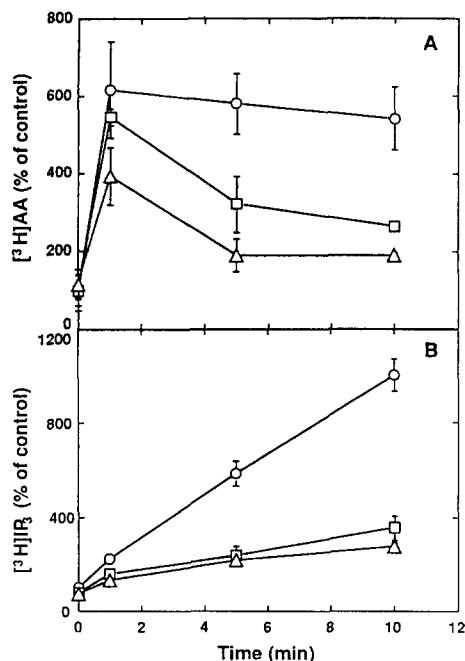
Fig. 4. Role of Ca^{2+} in SP-stimulated [³H]AA release and [³H]IP₃ formation in CHO-SPR cells. [³H]AA release (A) and [³H]IP₃ formation (B) were determined as described under Materials and Methods. Cells were preincubated in Ca^{2+}-containing medium (O), Ca^{2+}-free medium supplemented with 1 mM EGTA (□), or Ca^{2+}-free medium supplemented with 1 mM EGTA and 50 μM BAPTA/AM (△) for 15 min prior to the addition of 0.1 μM SP. Each point represents the mean ± SEM of triplicate determinations. The dpm/well at 0 min was 420 ± 210 (O), 460 ± 180 (□), and 530 ± 170 (△) for [³H]AA release; and 470 ± 30 (O), 380 ± 50 (□), 340 ± 40 (△) for [³H]IP₃ formation, respectively.

reaction mixture to prevent Ca^{2+} influx. As shown in Fig. 4A, although the response by chelating the intracellular Ca^{2+} with BAPTA was slightly less than that from cells exposed to EGTA alone, a significant [³H]AA release (56.4% of the release in the Ca^{2+}-containing medium) was still observed during the first 1 min. At 5 min after SP stimulation, the SP-stimulated [³H]AA release was only 18.8% of that from the cells incubated in the Ca^{2+}-containing medium. These results demonstrate that SP evoked the initial and rapid [³H]AA release in a Ca^{2+}-independent manner. On the other hand, extracellular Ca^{2+} is necessary to sustain [³H]AA release in the late phase. Figure 4B shows the effect of removal of extracellular Ca^{2+} or chelation of the intracellular Ca^{2+} on [³H]IP₃ accumulation. The SP-stimulated [³H]IP₃ accumulation was reduced markedly by chelation of the intracellular Ca^{2+} by BAPTA. [³H]IP₃ accumulation in cells incubated with BAPTA and EGTA exhibited 27.9, 23.9 and 19.5% of that in the cells incubated in the Ca^{2+}-containing medium at 1, 5, and 10 min, respectively.

Involvement of pertussis toxin-sensitive G-protein in [³H]AA release, but not in [³H]IP₃ formation,

following SP receptor activation. Because a direct activation of PLA₂ by a pertussis toxin-sensitive G-protein has been reported [20, 21], we next studied the involvement of a G-protein in SP-mediated stimulation of [³H]AA release. Six-hour pretreatment of CHO-SPR cells with pertussis toxin (200 ng/mL) impaired SP-stimulated [³H]AA release. This reduction by the toxin was 49.7 and 48.6% at 1 and 10 min, respectively, after stimulation with 0.1 μM SP (Table 1). When the cells were stimulated with 0.01 μM SP, the [³H]AA release was reduced further by 72.6% of the untreated cells at 1 min. Pertussis toxin did not inhibit the basal or ionomycin-stimulated release of [³H]AA (data not shown). On the other hand, [³H]IP₃ formation was not affected significantly by pretreatment of the cells with pertussis toxin under the same conditions. These results demonstrate that a pertussis toxin-sensitive G-protein could be involved in SP-stimulated [³H]-AA release, but not in the SP-stimulated [³H]IP₃ accumulation.

Effects of protein kinase C activator and inhibitor on SP-stimulated [³H]AA release and [³H]IP₃ formation. In addition to an increase in $[Ca^{2+}]_i$, phosphorylation of cytosolic PLA₂ is also known to increase the activity itself and to act synergistically with Ca^{2+} [18, 28, 29]. To evaluate this point, we studied the effect of TPA, an activator of protein kinase C, on [³H]AA release in CHO-SPR cells. As seen in Table 2, cells pretreated with 100 nM TPA alone did not show [³H]AA release over the basal level. However, TPA potentiated SP-stimulated [³H]AA release 2-fold (1241 ± 108 vs 566 ± 48% stimulated with SP alone), but the inactive phorbol ester 4α-PDD could not potentiate it. Furthermore, the protein kinase C inhibitor sphingosine [30] negated the potentiation of SP-stimulated [³H]AA release caused by TPA, but it failed to inhibit the SP-stimulated one. On the other hand, TPA had no significant effect on SP-stimulated [³H]IP₃ accumulation. These results demonstrate that the SP-stimulated [³H]AA is regulated by protein kinase C in a synergistic manner.

DISCUSSION

The present study has demonstrated that the activation of SP receptor stimulates both [³H]AA release and [³H]IP₃ formation in SP receptor cDNA-transfected CHO-SPR cells. PLA₂, which hydrolyzes AA from membrane phospholipids, can be classified into two distinct forms based on the apparent cellular localization: the secreted forms of PLA₂ and the cytosolic PLA₂ [28, 31]. Because the latter is activated by the translocation of the enzyme from the cytosol to the membrane by a physiological increase in $[Ca^{2+}]_i$, cytosolic PLA₂ is considered to mediate extracellular ligand-induced AA release [18, 29, 32–34]. The finding that a Ca^{2+} dependence of the SP-evoked [³H]AA release was prominent in the sustained phase (Fig. 4) is compatible with the assumption that translocation of cytosolic PLA₂ was induced by Ca^{2+} mobilization following [³H]IP₃ formation by SP. Together with an increase in $[Ca^{2+}]_i$, activation of PLA₂ and subsequent AA release can be regulated by phosphorylation in

Table 1. Effect of pretreatment with pertussis toxin on [^{3}H]AA release and [^{3}H]IP$_3$ formation in CHO-SPR cells elicited by SP

| | PT treatment | | |
Response	−	+	% of Decrease
[^{3}H]AA release (dpm)			
1 min	2860 ± 90	1440 ± 150	49.7
10 min	6980 ± 620	3590 ± 120	48.6
[^{3}H]IP$_3$ formation (dpm)			
1 min	570 ± 30	540 ± 20	5.3
10 min	4210 ± 300	4020 ± 350	4.5

CHO-SPR cells prelabeled with [^{3}H]AA or myo-[^{3}H]inositol were pretreated with or without 200 ng/mL of pertussis toxin for 6 hr at 37° before stimulation with 0.1 μM SP. Values represent means ± SEM (N = 3).

Table 2. Effect of protein kinase C modulators on SP-stimulated [^{3}H]AA release and [^{3}H]IP$_3$ formation

Addition	[^{3}H]AA release (%)	[^{3}H]IP$_3$ accumulation (%)
None	100 ± 8.5	100 ± 5.4
TPA	104 ± 27	130 ± 17
4α-PDD	135 ± 15	84 ± 5.0
Sphingosine	143 ± 27	81 ± 6.8
SP	566 ± 48	1038 ± 53
SP + TPA	1241 ± 108	1143 ± 75
SP + 4α-PDD	577 ± 110	993 ± 40
SP + sphingosine	446 ± 73	554 ± 112
SP + sphingosine + TPA	676 ± 38	843 ± 61

Confluent monolayers were prelabeled with [^{3}H]AA or myo-[^{3}H]inositol as described in the legend of Fig. 2. Cells were pretreated for 15 min with or without the protein kinase C inhibitor sphingosine (50 μM), and then with TPA (100 nM) or 4α-PDD (100 nM) for 10 min. The reaction was started by addition of 0.1 μM SP or medium. Data represent means ± SEM of experiments performed in triplicate. The dpm/well of the control was 450 ± 190 for [^{3}H]AA release and 470 ± 30 for [^{3}H]IP$_3$ formation.

response to extracellular ligands [19, 29]. Therefore, it was likely that the concomitant formation of diacylglycerol through activation of PLC by SP (Garcia M, unpublished observations) could activate protein kinase C or MAP kinase, which leads to the phosphorylation of PLA$_2$. In the present study, however, TPA produced a 2-fold potentiation of the SP-evoked [^{3}H]AA release, and the protein kinase C inhibitor sphingosine conversely blocked the TPA potentiation of [^{3}H]AA release but not the SP-induced one itself (Table 2). These results demonstrate that release of [^{3}H]AA in response to SP is mediated by Ca^{2+} mobilization, rather than by activation of protein kinase C, resulting from PI metabolism.

The EC$_{50}$ of SP for [^{3}H]AA release (Fig. 1) was similar to that for [^{3}H]IP$_3$ formation reported previously [25]. Both responses are composed temporally of two phases: an initial rapid burst detected as early as 15 sec, followed by a sustained phase largely, if not absolutely, dependent on

extracellular and intracellular Ca^{2+} (Fig. 3). In the initial phase, [^{3}H]AA release (56.4% of the release in the Ca^{2+}-containing medium) was affected less than [^{3}H]IP$_3$ formation (27.9%) by chelation of intracellular Ca^{2+} with BAPTA. Although CP-96,345 may interact with L-type Ca^{2+} channels in addition to being a potent SP receptor antagonist [26], we recently demonstrated that CHO-SPR cells do not have voltage-sensitive calcium channels [35]. This result negates the possibility that CP-96,345 inhibited SP-induced responses by interaction with L-type Ca^{2+} channels. While the release of [^{3}H]AA caused by SP was reduced by pretreatment with pertussis toxin, [^{3}H]IP$_3$ formation was not affected similarly (Table 1). These results demonstrate that activation of PLA$_2$ by SP at the early phase occurs in a Ca^{2+}-independent manner and may not depend on the prior activation of PLC. Although coupling of hormone receptors to PLA$_2$ via both pertussis toxin-sensitive and -insensitive G-proteins has been reported in several systems [20, 21, 36–39],

mechanisms for PLA_2 activation by a G-protein remain largely unknown at present. It is possible that SP may, in the first instance, stimulate [^3H]AA release by increasing the activity of the basal PLA_2 present in the membrane via a pertussis toxin-sensitive G-protein before more PLA_2 enzyme is translocated to the membrane, accompanied by an increase in [Ca^{2+}]$_i$ as mentioned above. Alternatively, since the reduction of [^3H]AA release by pertussis toxin was similar at 1 and 10 min, a G-protein may stimulate the release by reducing the calcium requirement of PLA_2. For example, in rat kidney cells, epidermal growth factor was reported to stimulate PLA_2 activity via pertussis toxin-sensitive G-protein without extracellular Ca^{2+} [40]. It is also conceivable that Ca^{2+}-independent PLA_2 stimulation can be mediated by intermediary proteins such as PLA_2-activating protein [41, 42]. Although the major pathway to release AA is by activation of PLA_2 [19], the contribution of other routes, such as the PLC/diacylglycerol lipase pathway [28, 43], in response to SP receptor activation remains a matter of further investigation.

A number of G-protein-linked receptors have been shown to regulate multiple effector pathways both when expressed endogenously and, more frequently, following transfection into heterologous systems [44]. We previously reported that the SP receptor is coupled to the stimulatory cascades of both PI hydrolysis and cyclic AMP formation in cDNA transfected CHO cells [25]. In the present study, we further demonstrated that SP stimulates [^3H]AA release via mechanisms that are mediated by a pertussis toxin-sensitive G-protein, dependent on extracellular Ca^{2+}, and enhanced by activation of protein kinase C. In this context, non-steroidal anti-inflammatory drugs recently have been shown to exert a direct spinal action by blocking the excessive sensitivity to pain (hyperalgesia) induced by the activation of spinal SP receptors in rats [45]. This observation was interpreted to mean that once the afferent barrage has generated a threshold effect upon neuronal function, the resulting activation of SP receptors causes the progressive generation of a pool of cytosolic arachidonic acid available for conversion by cyclooxygenase. However, the present study clearly indicates that cellular responses to a receptor are dependent on the other elements of signal transduction cascades expressed by the cell, and suggests that the integration of such information is likely to vary with cell type to define the final physiological response.

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