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Transfected D_2 Dopamine Receptors Mediate the Potentiation of Arachidonic Acid Release in Chinese Hamster Ovary Cells

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SUMMARY

A rat D_{2L} dopamine receptor, a splice variant of the D_2 receptor, has recently been cloned. When transfected into and stably expressed in Chinese hamster ovary cells, these receptors mediate the inhibition of both basal and forskolin-stimulated cAMP production, as previously described. We examined what role this receptor might play in the production of the second messenger arachidonic acid. The calcium ionophore A23187 stimulated the release of arachidonic acid, and this release of arachidonic acid was potentiated by dopamine in a concentration-dependent manner. Dopamine alone, however, had no effect on arachidonic acid release. Quinpirole, a D_2-selective agonist, augmented A23187-stimulated arachidonic acid release, and sulpiride, a D_2-selective antagonist, blocked this augmentation. cAMP analogs and agents that activate adenylyl cyclase were utilized in an attempt to overcome this dopamine effect. Forskolin, prostaglandin E_2, dibutyryl-cAMP, 8-(4-chlorophenylthio)-cAMP, and pertussis toxin all had no appreciable effect on either A23187-stimulated arachidonic acid release or the dopamine enhancement. Inhibition of protein kinase C using long term phorbol ester desensitization and pharmacological inhibitors diminished the dopamine potentiation of arachidonic acid release. These results suggest that the D_2 receptor may be increasing the release of arachidonic acid by a mechanism involving protein kinase C but independent of the D_2 receptor's inhibition of adenylyl cyclase.

Dopamine receptors belong to a class of neurotransmitter receptors that are linked to their signal transduction pathways via G proteins (1, 2). Dopamine receptors have been classified into two subtypes, based on their pharmacological and biochemical characteristics, D_1 and D_2 receptors (3). The D_2 receptor has classically been linked to the inhibition of adenylyl cyclase (2, 3), and it is particularly noteworthy because of its role in schizophrenia and neuroleptic drug action (4) and its role in the regulation of prolactin secretion from the pituitary (5). The rat D_2 dopamine receptor has been recently cloned by several groups and appears to exist in two major isoforms, differing by a 29-amino acid sequence within the putative third cytoplasmic loop of the receptor (6, 7). The tissue distribution of these two isoforms is similar, but the longer form, the D_{2L} receptor, appears to be the predominant form expressed in most tissues (7). At this time, there is no evidence that these two isoforms differ in their radioligand-binding properties or in their coupling to the inhibition of adenylyl cyclase.

In addition to the inhibition of adenylyl cyclase, the D_2 receptor has been previously linked to numerous signaling pathways, including inhibition of phospholipase C and subsequent inositol phospholipid turnover, activation of potassium channels, and inhibition of calcium channels (8). Recently, it has also been demonstrated that the D_2 receptor mediates the inhibition of free arachidonic acid release from anterior pituitary cells, and this phenomenon occurs independently of the adenylyl cyclase-cAMP pathway (9).

Arachidonic acid release via the phospholipase A_2 pathway has been shown to be a signal transduction pathway for numerous neurotransmitter, hormone, and peptide receptors in various tissues and cell lines (10). Arachidonic acid and its eicosanoid metabolites are biologically ubiquitous and have a multitude of physiological and pathophysiological actions, particularly in the nervous system (11). We have recently shown that adrenergic and serotonergic receptors (12, 13) mediate the release of arachidonic acid from neurons in primary culture. In this paper, we have utilized the recently cloned D_{2L} receptor, transfected into and stably expressed in CHO cells, to study the effect of the D_2 receptor on the release of arachidonic acid. We have demonstrated that the D_2 receptor mediates the potentiation of arachidonic acid release through a mechanism involving protein kinase C but independent of the adenylyl cyclase cascade.

ABBREVIATIONS: G protein, GTP-binding protein; CHO, Chinese hamster ovary; PMA, phorbol 12-myristate, 13-acetate; Sp-cAMPS, adenosine-3′,5′-cyclic monophosphothilate, Sp isomer; Rp cAMPS, adenosine-3′,5′-cyclic monophosphothiolate, Rp isomer; BSA, bovine serum albumin; H-7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hyrochloride.

Experimental Procedures

Materials. [5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid was purchased from New England Nuclear. PMA, staurosporine, and A23187 were purchased from Calbiochem (San Diego, CA); pertussis toxin from List Biological Laboratories (Campbell, CA); recombinant melittin from Peninsula Laboratories (Belmont, CA); dopamine, spiperone, quinpirole, apomorphine, haloperidol, and sulpiride from Research Biochemicals, Inc. (Natick, MA); Rp-cAMPS and Sp-cAMPS from Biolog Life Science Institute (La Jolla, CA); and H-7 and W-7 from Biomol (Plymouth Meeting, PA). All other reagents were from Sigma. α Minimal essential medium was from Whittaker Bioproducts, Inc. (Walkersville, MD) and Eagles' no. 2 medium was from the National Institutes of Health Media Unit.

Stable transfection of D_{2L} clone into CHO cells. The D_{2L} cDNA insert was excised from pBluescript with *Xho*I and *Xba*I and ligated into the corresponding restriction sites in pcD-SRα (14) containing a modified polylinker. CHO-K1 cells were transfected with 30 μg of pSRα-D_{2L} and 3 μg of pMAM-Neo (Invitrogen) by the $CaPO_4$ precipitation method, as described (15). Stable transfectants were selected in the presence of 500 μg/ml G418 (GIBCO) and were subcloned by limiting dilution. CHO cells were maintained in Ham's F12 medium plus 10% fetal calf serum and 500 μg/ml G418. D_2 receptor expression was quantified in CHO cell membranes by saturation binding with [^{3}H] methylspiperone, as previously described (16). Cell lines expressing 1.2 pmol/mg of protein were utilized for this study. The ligand-binding properties of this receptor have previously been described (7).

Cell culture of CHO cells transfected with and stably expressing the cloned D_{2L} dopamine receptor (CHO D_{2L} cells). Cells were maintained in α minimum essential medium with 10% fetal calf serum. Two days before experimentation, cells were plated, at 100,000 cells/well, in 24-well Costar plastic culture plates (Becton Dickinson, Oxnard, CA).

Measurement of cAMP accumulation in CHO cells. CHO cells were grown to confluence, and the growth medium was replaced with 250 μl of Eagles' no. 2 medium containing 1 mM 3-isobutyl-1-methylxanthine. Experimental agents were added. The reaction was stopped after 5 min with 250 μl of an ice-cold solution containing 0.1 N HCl and 1 mM $CaCl_2$. The accumulation of cAMP was measured by radioimmunoassay, as previously described (17).

Measurement of [^{3}H]arachidonic acid release. CHO cells were incubated with [^{3}H]arachidonic acid (0.20 μCi/well) to isotopic equilibrium (18–24 hr). Before the addition of experimental agents, the cells were washed twice with 0.5 ml of serum-free Eagles' no. 2 medium supplemented with 0.2% fatty acid-free BSA. The BSA was used to trap free arachidonic acid in the extracellular medium. The experimental agents were added in a final volume of 0.5 ml, and the reaction was allowed to proceed for 15 min at 37°. The reaction was stopped by removal of the incubation medium, which was then centrifuged at 12,000 × *g* for 1 min to remove nonadherent cells. Four hundred microliters of the supernatant were removed, and released [^{3}H]arachidonic acid was measured with a liquid scintillation counter. High performance liquid chromatographic analysis revealed that greater than 99% of released radioactivity in the presence of 0.2% BSA was attributable to arachidonic acid. Cell viability at the end of the incubation period with A23187 was confirmed by the trypan blue exclusion test.

Data analysis. All experiments were done in triplicate, with data being presented as the means of at least three experiments ± standard error. Statistical analysis was done by analysis of variance, with subsequent calculation of Bonferroni's *p* value (InStat by GraphPAD Software, San Diego, CA). EC_{50} and IC_{50} values were calculated using InPlot 3.0 (GraphPAD Software).

Results

Dopamine inhibits the production of cAMP in CHO D_{2L} cells. Because the D_2 dopamine receptor has recently been shown to reduce free arachidonic acid release from anterior pituitary cells (9), we examined what role the D_2 receptor might have on the release of arachidonic acid in CHO cells stably expressing the D_2 dopamine receptor (CHO D_{2L} cells). To assess the functional association between the D_2 dopamine receptor and adenylyl cyclase in the CHO D_{2L} cells, we examined the effect of dopamine on forskolin-stimulated cAMP production. Dopamine inhibited the forskolin-stimulated generation of cAMP in a concentration-dependent manner (IC_{50} = 5.5 nM), comparable to that which has been previously demonstrated (Fig. 1) (18). Dopamine similarly inhibited prostaglandin E_2-stimulated cAMP production and modestly inhibited the production of cAMP from unstimulated CHO D_{2L} cells (data not shown). This demonstrates that, in transfected CHO D_{2L} cells, the D_2 dopaminergic receptor coupled normally to adenylyl cyclase inhibition.

Dopamine potentiates A23187-stimulated release of arachidonic acid. The effect of dopamine on arachidonic acid release in CHO D_{2L} cells was then examined. Dopamine did not inhibit the release of arachidonic acid from CHO D_{2L} cells, nor did it by itself stimulate arachidonic acid release. Dopamine instead caused a potentiation of arachidonic acid release stimulated by activators of phospholipase A_2 in CHO D_{2L} cells. A23187, the divalent cation ionophore (19), has been shown to release arachidonic acid in a number of cells (20, 21). Dopamine enhanced the A23187-stimulated release of arachidonic acid in a concentration-dependent manner (EC_{50} = 29 nM) (Fig. 2).

In addition to A23187, melittin and PMA are known to stimulate phospholipase A_2 activity and arachidonic acid release in a variety of cells (22–24). They were subsequently examined to determine whether their ability to stimulate arachidonic acid release was also enhanced by dopamine. Melittin-stimulated release of arachidonic acid was increased by dopamine, although not as effectively as with A23187 stimulation (Fig. 3A). PMA (100 nM) alone minimally stimulated the release of arachidonic acid, but this response was also enhanced by dopamine (Fig. 3B). Higher concentrations of PMA (up to 10 μM), which alone caused a more robust release of arachidonic acid, were also amplified by dopamine (data not shown). The dopamine-mediated potentiation of both melittin- and PMA-stimulated arachidonic acid release was inhibited by the D_2 dopamine receptor antagonist spiperone. These data indicate that dopamine mediates the augmentation of arachidonic acid release stimulated by agents that activate phospholipase A_2 in

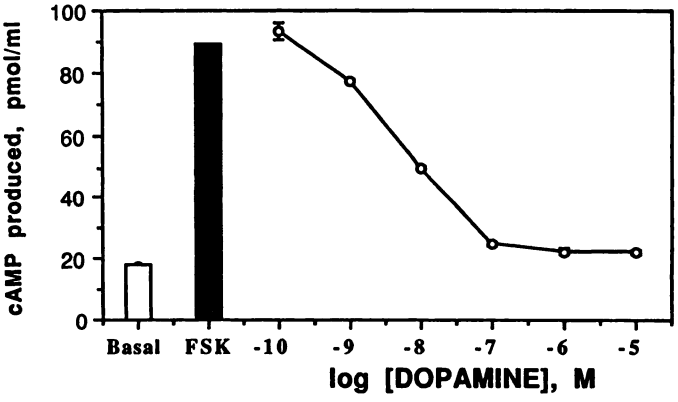

Fig. 1. Dopamine inhibits forskolin (*FSK*)-stimulated cAMP production in CHO D_{2L} cells. The IC_{50} of this inhibition is 5.5 nM. Cells were incubated with 100 nM forskolin for 5 min. Data are means ± standard errors of three experiments performed in triplicate.

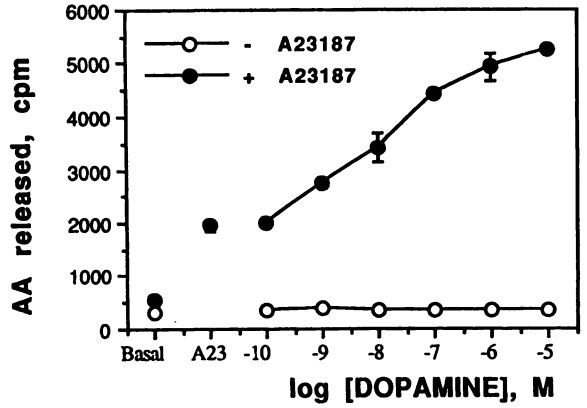

Fig. 2. Dopamine potentiates the release of arachidonic acid (*AA*) from A23187-stimulated CHO D_{2L} cells. Data are reported as cpm of [^{3}H] arachidonic acid released. ○, Cells incubated without A23187; ●, cells stimulated with A23187 (10 μM). Cells were incubated with A23187 for 15 min. Dopamine potentiation of arachidonic acid release occurs in a concentration-dependent manner, with an EC_{50} of 29 nM. Data are means ± standard errors of three experiments performed in triplicate.

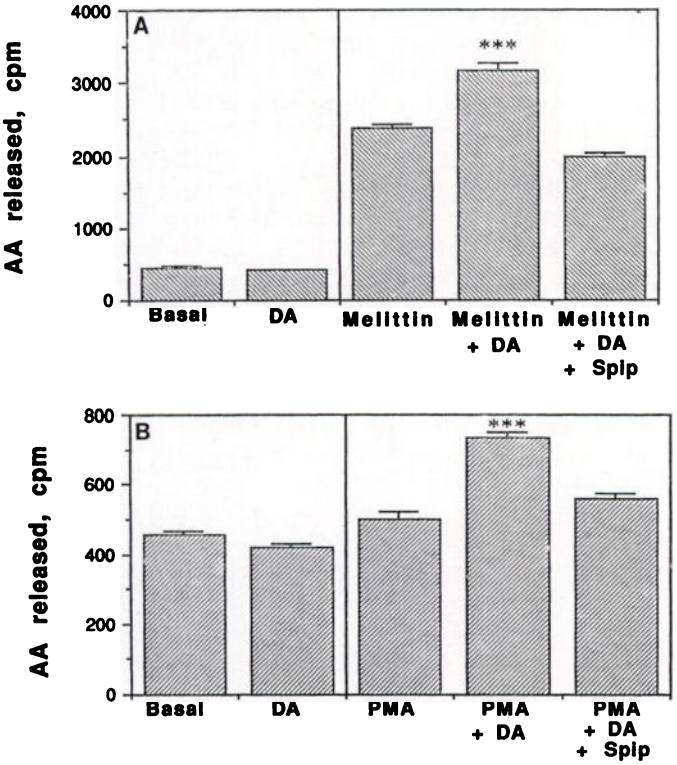

Fig. 3. Dopamine (*DA*) potentiates the release of arachidonic acid (*AA*) stimulated by melittin (A) and PMA (B) in CHO D_{2L} cells. Data are reported as cpm of [^{3}H]arachidonic acid released. Cells were stimulated with melittin (1 μg/ml, 15 min) (A) or PMA (100 nM, 60 min) (B) and amplified with dopamine (1 μM). Spiperone (*Spip*), at 1 μM, abolishes the dopamine-mediated amplification of arachidonic acid release. Data are means ± standard errors of three experiments performed in triplicate. ***, Significant increase, compared with that stimulated by melittin (A) or PMA (B) alone, Bonferroni's $p < 0.001$.

CHO D_{2L} cells. Preliminary results showed that dopamine also enhanced A23187-stimulated arachidonic acid release in CHO cells transfected with and stably expressing the D_{2S} receptor, the shorter splice variant of the D_{2L} receptor (6, 7) (data not shown).

To demonstrate that the dopamine-mediated potentiation of arachidonic acid release was neither a native function of the CHO cells nor a byproduct of the transfection procedure, we used CHO cells transfected with and stably expressing another neurotransmitter receptor (the m5 muscarinic receptor). Dopamine failed to enhance the release of arachidonic acid from CHO cells transfected with the m5 receptor (data not shown).

Dopamine agonists potentiate A23187-stimulated release of arachidonic acid and dopamine antagonists block this effect. To further investigate the hypothesis that the dopamine-mediated potentiation of A23187-stimulated arachidonic acid release was occurring via the D_2 dopaminergic receptor, dopamine agonists were used to mimic this effect and antagonists to block it. The nonselective D_1/D_2 dopamine receptor agonist apomorphine and the D_2-specific agonist quinpirole (LY 171555) both amplified A23187-stimulated arachidonic acid release, with a potency comparable to that of dopamine (Fig. 4A). Additionally, both of these responses were inhibited by the D_2 antagonist spiperone (data not shown). Haloperidol and (−)-sulpiride, D_2-selective antagonists, both inhibited the dopamine-mediated enhancement of A23187-stimulated arachidonic acid release, in a concentration-dependent manner (Fig. 4B).

Potentiation of arachidonic acid release by dopamine

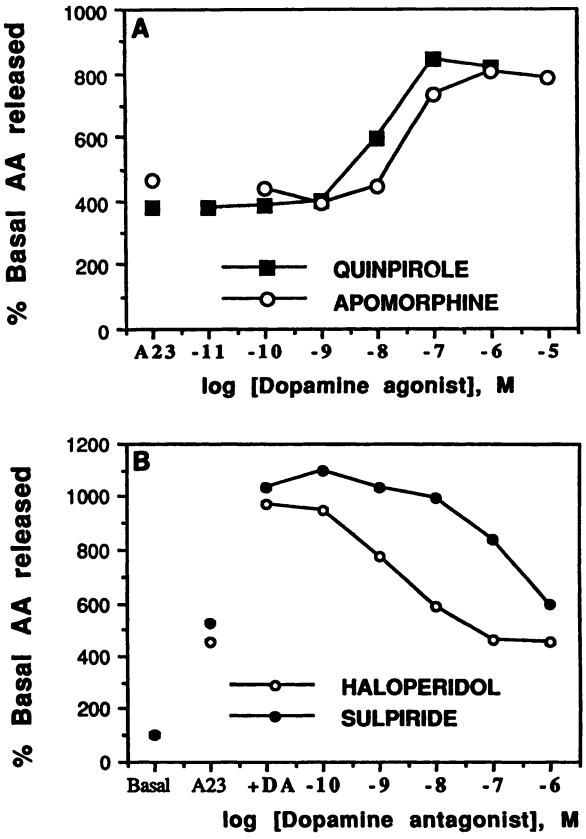

Fig. 4. Dopamine agonists potentiate A23187-stimulated arachidonic acid (*AA*) release (A) and dopamine antagonists inhibit the potentiation of arachidonic acid release (B) in CHO D_{2L} cells. Data are reported as percentage of basal [^{3}H]arachidonic acid release. A, ○, Apomorphine; ●, quinpirole. Cells were incubated with A23187 (*A23*), (1 μM) and appropriate concentrations of agonist. EC_{50} values are 20 nM for apomorphine and 11 nM for quinpirole. B, ○, Sulpiride; ●, haloperidol. Cells were incubated with A23187, dopamine (*DA*), and appropriate antagonist. IC_{50} values are 1.6 nM for haloperidol and 130 nM for sulpiride. Data represent means ± standard errors of three experiments performed in triplicate.

occurs independently of adenylyl cyclase and cAMP-dependent protein kinase. Dopamine-mediated inhibition of adenylyl cyclase and reduction of cellular levels of cAMP might provide one possible explanation for the dopamine-mediated augmentation of arachidonic acid release in CHO D_{2L} cells. It has been recently shown that cAMP can inhibit arachidonic acid release in both endothelial cells and astrocytes (25). Consistent with this model, a reduction of cAMP, mediated by the D_2 receptor, could possibly increase (by disinhibition) arachidonic acid release in CHO D_{2L} cells. To examine the role of cAMP in the potentiation of arachidonic acid release, we used agents that stimulate adenylyl cyclase in CHO cells and agents that substitute for cellular cAMP, in an attempt to overcome any inhibition by the D_2 receptor of the adenylyl cyclase-cAMP cascade. These agents did not diminish A23187-stimulated arachidonic acid release in the absence of dopamine, nor were they able to overcome the potentiation by dopamine of arachidonic acid release (Table 1).

Further evidence for the noninvolvement of the adenylyl cyclase cascade in the D_2 receptor-mediated potentiation of arachidonic acid release was obtained using Sp-cAMPS, an activator of protein kinase A (26) and Rp-cAMPS, an inhibitor of protein kinase A (27). Rp-cAMPS would also amplify A23187-stimulated arachidonic acid release if protein kinase A inhibition were involved in this potentiation. Rp-cAMPS, however, failed to increase the A23187-mediated arachidonic acid release (Fig. 5). Sp-cAMPS, the cAMP analog and activator of protein kinase A, failed to inhibit the dopamine-mediated augmentation of A23187-stimulated arachidonic acid release, consistent with data presented above.

The D_2 receptor couples to adenylyl cyclase inhibition via the G protein G_i (1, 2). Pertussis toxin is known to inhibit the function of G_i, thereby blocking the ability of the D_2 receptor to inhibit adenylyl cyclase (1). At concentrations up to 50 ng/ml, pertussis toxin completely abolished the inhibition by dopamine of cAMP production in CHO D_{2L} cells but was unable to inhibit the D_2-mediated potentiation of arachidonic acid release (Table 2). These findings further demonstrate that the inhibition of cAMP production by the D_2 receptor is independent of the mechanism by which the D_2 receptor can enhance the release of arachidonic acid.

Inhibition of protein kinase C diminishes the dopamine-mediated potentiation of arachidonic acid release. Protein kinase C is known to have a stimulatory effect on phospholipase A_2 and on arachidonic acid release (23, 24). We, therefore, examined the role of protein kinase C in the dopamine-mediated potentiation of arachidonic acid release in CHO D_{2L} cells. Long term treatment with phorbol ester is known to desensitize protein kinase C activity (28). When CHO D_{2L} cells were preincubated with PMA for 24 hr, both A23187-stimulated and dopamine-enhanced A23187-stimulated arachidonic acid release were decreased (Fig. 6). However, the dopamine-enhanced A23187-stimulated arachidonic acid release was inhibited to a greater extent than that stimulated by A23187 alone. Similarly, dopamine-augmented melittin-stimulated arachidonic acid release was inhibited by 46% ($p < 0.001$) after 24-hr PMA treatment, whereas melittin-stimulated arachidonic acid release (in the absence of dopamine) was not significantly inhibited by long term PMA treatment.

The protein kinase C inhibitors staurosporine, H-7, and sphingosine (29) were also utilized, and all inhibited the D_2-mediated potentiation of A23187-stimulated arachidonic acid release (Table 3). None of these agents, however, significantly inhibited A23187-stimulated arachidonic acid release in the absence of dopamine in CHO D_{2L} cells (data not shown). Also, W-7, a calmodulin antagonist and, at higher concentrations, protein kinase C antagonist (29), blocked dopamine-enhanced

TABLE 1

Agents that increase adenylyl cyclase activity or substitute for intracellular cAMP do not modify the potentiation effect of dopamine on A23187-stimulated arachidonic acid release in CHO D_{2L} cells

Cells were pretreated with cAMP-elevating agents or cAMP analogs for 15 min, to allow cAMP (and its effects) to accumulate, and then stimulated with A23187 (10 μM), in the presence or absence of dopamine (1 μM). [^{3}H]Arachidonic acid released was measured as cpm. PGE_2, prostaglandin E_2; FSK, forskolin; CPT-cAMP, 8-(4-chlorophenylthio)-cAMP; dB-cAMP, dibutyryl-cAMP. Cells were incubated with the aforementioned agents, with or without dopamine, for 15 min before stimulation with A23187 for 15 min. Basal arachidonic acid release is 329 ± 15 cpm. Data represent means ± standard errors of three experiments performed in triplicate.

	Arachidonic acid	
	−Dopamine	+Dopamine
	cpm	
A23187 (10 μM)	1367 ± 80	2699 ± 62
A23187 + PGE_2 (1 μM)	1303 ± 17	2939 ± 89
A23187 + FSK (100 nM)	1650 ± 33	2693 ± 102
A23187 + CPT-cAMP (10 μM)	1172 ± 9	2347 ± 57
A23187 + dB-cAMP (10 μM)	1132 ± 16	2348 ± 70

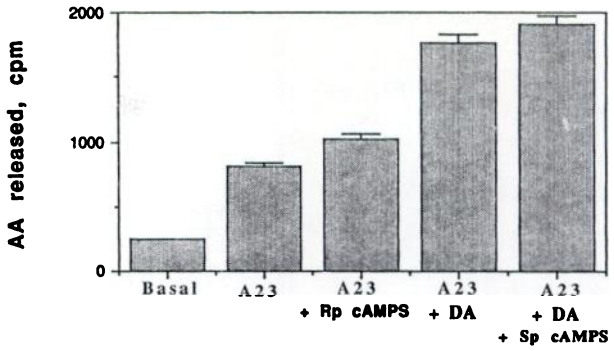

Fig. 5. Rp-cAMPS does not potentiate A23187-stimulated arachidonic acid (*AA*) release; Sp-cAMPS does not inhibit dopamine (*DA*) potentiation of arachidonic acid release in CHO D_{2L} cells. Data are reported as cpm of [^{3}H]arachidonic acid released. Cells were incubated with appropriate agents at the following concentrations: A23187 (*A23*), 10 μM; dopamine, 1 μM; Rp-cAMPS, 10 μM; and Sp-cAMPS, 10 μM. Cells were incubated with A23187 for 15 min, following incubation with Rp-cAMPS and Sp-cAMPS for 15 min (where appropriate). Data are means ± standard errors of three experiments performed in triplicate.

TABLE 2

Effect of pertussis toxin on dopamine inhibition of cAMP production and dopamine potentiation of arachidonic acid release

Cells were pretreated with pertussis toxin (PTX) at appropriate concentrations for 18 hr, before treatment with dopamine and either forskolin or A23187. Cells were stimulated with forskolin (for cAMP production experiments) or A23187 (for arachidonic acid release experiments). Arachidonic acid release and cAMP production were measured as described in Experimental Procedures. Data represent means ± standard errors of three experiments performed in triplicate.

	cAMP produced	Arachidonic acid released
	pmol/ml	cpm
Basal	13.6 ± 0.5	534 ± 27
Forskolin (500 nM)	78.7 ± 1.0	
A23187 (10 μM)		1336 ± 15
+Dopamine (1 μM)	11.8 ± 0.6	2033 ± 18
+Dopamine, +PTX (0.5 ng/ml)	52.9 ± 3.6	2134 ± 47
+Dopamine, +PTX (50 ng/ml)	73.2 ± 3.2	2001 ± 33

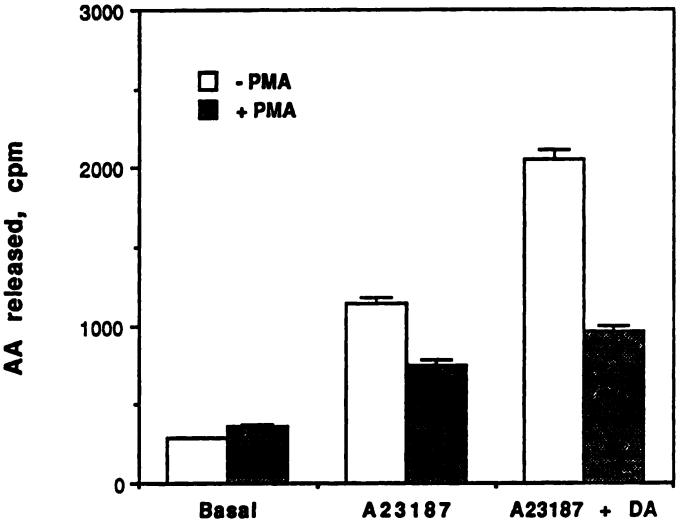

Fig. 6. Long term PMA treatment inhibits dopamine (*DA*)-potentiated A23187-stimulated arachidonic acid (*AA*) release more than it inhibits A23187-stimulated arachidonic acid release in CHO D_{2L} cells. Data are reported as cpm of [^{3}H]arachidonic acid released. □, Cells not treated with PMA; ■, cells treated with PMA (1 μM) for 24 hr. Appropriate cells were then treated with A23187 (10 μM) and dopamine (1 μM). Data are means ± standard errors of three experiments performed in triplicate.

TABLE 3

Effect of protein kinase C inhibitors on A23187-stimulated arachidonic acid release, with or without dopamine, in CHO D_{2L} cells

Cells were pretreated with protein kinase C antagonists for the times indicated below, and then dopamine (1 μM) was added to those cells that were to receive it, followed by the addition of A23187 (10 μM). Data are reported as percentage of inhibition of maximal stimulated arachidonic acid release. Staurosporine mildly, but not significantly, increased A23187-stimulated arachidonic acid release in this experiment. Agents were preincubated for the following times: staurosporine and H-7, 45 min; sphingosine, 15 min. Data represent means ± standard errors of three experiments performed in triplicate and were subjected to analysis of variance, with calculation of Bonferroni *p* values.

	Inhibition of maximum arachidonic acid release	
	−Dopamine	+Dopamine
	%	
H-7 (10 μM)	2 ± 4.6[a]	38 ± 2.9[b]
Sphingosine (5 μM)	18 ± 1.2[a]	69 ± 3.1[b]
Staurosporine (1 μM)	+6 ± 3.9[a]	54 ± 1.8[b]

[a] Inhibition not significant.
[b] $p < 0.001$.

A23187-stimulated arachidonic acid release by 91% ($p < 0.001$). These data suggest a role for protein kinase C in the D_2 receptor-mediated amplification of arachidonic acid release in CHO D_{2L} cells.

Discussion

The D_2 dopamine receptor, when transfected into and stably expressed in CHO cells, not only inhibited the production of cAMP but also potentiated the release of arachidonic acid. A23187 and melittin, two activators of phospholipase A_2 (22, 23), were, therefore, utilized to stimulate arachidonic acid release. A23187, a divalent cation ionophore, stimulates the release of arachidonic acid presumably through activation of phospholipase A_2, a calcium-sensitive enzyme (20, 21). Melittin also stimulates arachidonic acid release, by a mechanism involving direct activation of phospholipase A_2 (22).

Pharmacological studies verified the functional coupling of this D_2 receptor clone to the inhibition of cAMP production (6, 7). The potentiation of arachidonic acid release exhibited similar pharmacology. Apomorphine, a nonselective dopamine agonist, and quinpirole, a D_2-selective dopamine agonist, mimicked the amplification effect, whereas haloperidol and sulpiride, D_2-selective dopamine antagonists, blocked this response.

Previous studies have demonstrated that cAMP can inhibit the release of arachidonic acid in endothelial cells and astrocytes (25). In contrast, in CHO D_{2L} cells the mechanism of the D_2 receptor potentiation of arachidonic acid release appears to be independent of inhibition of the adenylyl cyclase-cAMP pathway. None of the various cAMP analogs or cAMP-elevating agents were able to overcome the dopamine-mediated amplification of A23187-stimulated release of arachidonic acid in CHO D_{2L} cells. In these studies, the D_2 receptor lowers basal levels of cAMP, possibly removing a tonic inhibition of arachidonic acid release. This was tested by inhibiting the adenylyl cyclase cascade distally, at the level of protein kinase A. When the protein kinase A inhibitor Rp-cAMPS (27) was added, no effect on arachidonic acid release was observed, providing additional evidence for the noninvolvement of the cAMP pathway (at the level of protein kinase A) in the D_2 receptor-mediated potentiation of arachidonic acid release. Furthermore, dopamine inhibited cAMP production with a potency greater than that with which dopamine increased arachidonic acid release (IC_{50} for cAMP inhibition, 5.5 nM; EC_{50} for arachidonic acid potentiation, 29 nM). The D_2 receptor-mediated inhibition of adenylyl cyclase involves the G protein G_i (1, 2), and treatment with pertussis toxin is known to abolish the receptor-mediated activation of G_i (1). Treatment of CHO D_{2L} cells with pertussis toxin had no effect on the potentiation of arachidonic acid release by dopamine, suggesting that G_i is not involved in this response. When transfected and stably expressed in CHO cells, the m2 muscarinic receptor, which also couples to the G protein G_i (1), similarly potentiates the release of arachidonic acid.[1] The D_2 receptor-mediated potentiation of arachidonic acid release appears to be a novel signal transduction pathway that is independent of the adenylyl cyclase cascade.

Protein kinase C has been shown to be involved in phospholipase A_2 activation and in the release of arachidonic acid (23, 24). In CHO D_{2L} cells, desensitization of protein kinase C by long term treatment with phorbol ester (28) inhibited both dopamine-potentiated A23187-stimulated arachidonic acid release and nonpotentiated A23187-stimulated arachidonic acid release. However, the effect on the dopamine-potentiated arachidonic acid release was greater. Similarly, pharmacological inhibitors of protein kinase C (staurosporine, sphingosine, and H-7) also reduced dopamine-potentiated, but not the nonpotentiated, A23187-stimulated arachidonic acid release. The mechanism by which protein kinase C may be involved is thus far unclear. It does not appear that the D_2 receptor increases diacylglycerol via phosphatidylinositol-4,5-bisphosphate hydrolysis by phospholipase C, inasmuch as dopamine fails to stimulate the concomitant production of inositol phosphates in CHO D_{2L} cells. After preincubation with [^{3}H]inositol, dopamine (10 μM)-stimulated total inositol phosphate release (342 ± 11 cpm) was no different from basal inositol phosphate release (346 ± 8 cpm). Nonetheless, the aforementioned results suggest

[1] C. C. Felder, R. Y. Kanterman, and J. Axelrod, unpublished observations.

that protein kinase C plays a role in the dopamine-mediated potentiation of arachidonic acid release in CHO D_{2L} cells.

D_2 receptor-mediated inhibition of cAMP production and inositol phosphate generation, as well as arachidonic acid release, have previously been demonstrated in pituitary cells (8, 9). We have also found that, in AtT-20 pituitary cells transfected with this same D_{2L} receptor, dopamine inhibits melittin-stimulated arachidonic acid release.[2] These findings illustrate that a single receptor, in this case a cloned D_2 dopamine receptor, may generate multiple biological signals within a given cell. Multiple signals generated from a single receptor gene product have been shown for the m1 and m3 muscarinic receptors (30, 31). In addition, it appears that the same receptor may have different, even opposite, effects, depending on the cell in which it is expressed. This is illustrated by the D_2 receptor-mediated inhibition of arachidonic acid release in AtT-20 cells and D_2 receptor-mediated potentiation of arachidonic acid release in CHO cells. Differential coupling, dependent on the cell into which the receptor is transfected and expressed, has recently been demonstrated for the D_2 receptor. When expressed in LtK^- fibroblasts, the D_2 receptor stimulated inositol phosphate production and intracellular calcium accumulation; in GH_4C_1 cells, the D_2 receptor mediated a decrease in intracellular calcium, with no effect on inositol phosphate production (32). This phenomenon may be a result of differential processing, trafficking, or placement of the D_2 receptor within a cell membrane or the differential availability of effector enzymes, ion channels, or G proteins among distinct cell types.

[2] C. C. Felder, R. Y. Kanterman, L. C. Mahan, and J. Axelrod, unpublished observations.

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