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Authors
Marzo, Vincenzo
Piomelli, Daniele

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Rapid Communication

Participation of Prostaglandin E_2 in Dopamine D_2 Receptor-Dependent Potentiation of Arachidonic Acid Release

Vincenzo Di Marzo and *Daniele Piomelli

Istituto per la Chimica di Molecole di Interesse Biologico, CNR, Arco Felice, Italy; and
**Unité de Neurobiologie et Pharmacologie de l'INSERM (U. 109), Paris, France*

Abstract: Stimulation of dopamine D_2 receptors potentiates Ca^{2+} ionophore- or ATP-induced arachidonic acid (AA) release in D_2 receptor cDNA-transfected Chinese hamster ovary (CHO) cells [CHO(D_2)]. By using a combination of chromatographic, biochemical, and radioimmunochemical techniques, we show here that prostaglandin (PG) E_2 is a major product of AA metabolism in CHO(D_2) cells stimulated with the Ca^{2+} ionophore A23187. Formation of this PG was markedly increased by the concomitant application of quinpirole, a D_2 receptor agonist. In addition, PGE_2 enhanced D_2-dependent amplification of AA release, either when it was added (EC_{50} = 100 n*M*) or when it was produced endogenously, as shown by experiments carried out with the cyclooxygenase inhibitor indomethacin. The results suggest that PGE_2 may participate in D_2 receptor-mediated potentiation of AA release in CHO(D_2) cells. They also support a functional role for this PG in the modulation of dopaminergic transmission in areas of the CNS, such as amygdala and hypothalamus, where high levels of both PGE_2 and dopamine D_2 receptors are found. **Key Words:** Transfected cells—Prostaglandin receptors—D_2 receptors—Arachidonic acid. **Di Marzo V. and Piomelli D.** Participation of prostaglandin E_2 in dopamine D_2 receptor-dependent potentiation of arachidonic acid release. *J. Neurochem.* **59,** 379–382 (1992).

Arachidonic acid (AA) and its metabolites are thought to serve two distinct signaling functions in neural tissue. They may act as second messengers by modulating intracellularly the activities of ion channels and protein kinases, or they may be released from the neuron of origin and act as extracellular local mediators by binding to guanine nucleotide binding regulatory protein (G protein)-coupled membrane receptors of neighboring cells (Wolfe and Coceani, 1979; Axelrod et al., 1988; Piomelli and Greengard, 1990). Both lipoxygenase and cyclooxygenase metabolites have been proposed to exert such extracellular actions, which may be similar to the paracrine functions attributed to biogenic amines and neuropeptides (for review, see North, 1989). For example, the cyclooxygenase product prostaglandin (PG) E_2 is thought to participate in α_1-adrenergic modulation of brain cyclic AMP (cAMP) levels, presumably by binding to neural EP_2-type receptors positively linked to adenylyl cyclase activity (Partington et al., 1980; Schaad et al., 1987; Weidenfeld et al., 1992).

Evidence indicates that stimulation of dopamine D_2 receptors enhances release and metabolism of AA and that activation of this signaling pathway may underlie some physiological D_2 responses. In the median eminence, selective inhibitors of epoxygenase-mediated AA metabolism prevent D_2 receptor-mediated release of somatostatin from hypothalamic nerve terminals, whereas application of 8,9-epoxyeicosatrienoic acid, an endogenous epoxygenase metabolite of AA, stimulates somatostatin release (Junier et al., 1990). In kidney renal medulla, a D_2-like receptor is linked to formation of PGE_2 (Huo et al., 1991), and in rat brain synaptosomes, dopamine (100 μM) was reported to stimulate formation of immunoreactivity PGE_2 (i-PGE_2) (Hillier et al., 1976). Finally, in Chinese hamster ovary (CHO) cells transfected with D_2 receptor DNA [CHO(D_2)], D_2 agonists enhance the release of AA potently, when such release has been initiated by stimulating constitutive Ca^{2+}-mobilizing receptors or by applying Ca^{2+} ionophores (Felder et al., 1991; Kanterman et al., 1991; Piomelli et al., 1991).

Here, we show that PGE_2 is a major metabolite of AA in stimulated CHO(D_2) cells and that this PG enhances, in turn, D_2 receptor-dependent potentiation of AA release. The results support a functional role for PGE_2 as a paracrine messenger induced by dopaminergic activation.

EXPERIMENTAL PROCEDURES

Cell culture and [^{3}H]AA release experiments

CHO cells were transfected with rat D_{2A} receptor (also termed D_{2L}) cDNA as previously described (Sokoloff et al., 1990). Transfected clones were maintained in monolayer culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% serum calf serum (GIBCO) at 37°C

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Address correspondence and reprint requests to Dr. D. Piomelli at Unité de Neurobiologie et Pharmacologie de l'INSERM (U. 109), 2ter Rue d'Alésia, Paris 75014, France.
Abbreviations used: AA, arachidonic acid; BSA, bovine serum albumin; cAMP, cyclic AMP; CHO, Chinese hamster ovary; CHO(D_2), Chinese hamster ovary cells transfected with D_2 receptor cDNA; DMEM, Dulbecco's modified Eagle's medium; G protein, guanine nucleotide binding regulatory protein; i-PGE_2, immunoreactive prostaglandin E_2; PG, prostaglandin; RIA, radioimmunoassay.

in 5% CO_2. $CHO(D_2)$ cells expressed 1.3×10^5 D_2 receptors per cell. Cells (24-well plates) were labeled by incubation with [^{3}H]AA (200–220 Ci/mmol, 0.25 mCi/ml; Amersham) in DMEM (1 ml) containing 0.2% bovine serum albumin (BSA; essentially fatty acid free; Sigma) for 3 h at 37°C. We chose these prelabeling conditions rather than overnight incubation with [^{3}H]AA (Kanterman et al., 1991) because the latter was found to cause a complete loss of the AA response to PGE_2. To eliminate unincorporated radioactivity, cells were washed with 1 ml DMEM plus BSA, before they were incubated for 30 min at 37°C in 1 ml of DMEM containing final concentrations of the appropriate drugs. BSA was omitted from drug incubations to maximize intracellular AA metabolism and to prevent binding to endogenously formed PGE_2. [^{3}H]AA release was determined by liquid scintillation counting in samples (0.5 ml) of the incubation medium. Statistical comparisons between mean ± SD values were carried out using analysis of variance.

Analytical methods

For analytical purposes, $CHO(D_2)$ cells were cultured to confluence in 100-mm-diameter tissue culture dishes, and labelled by incubation with [^{3}H]AA as described above. After washing and incubation with the appropriate drugs (30 min), samples (5 ml) of bath solutions were applied to a prewashed C18 Sep-Pak column (Waters Associates) and eluted with 100% methanol. Methanol fractions were brought to dryness under vacuum, and the residues were resuspended in 0.1 ml of acetonitrile for HPLC analysis. Reversed-phase HPLC was carried out using a Deltapack column (150 × 4.5 mm; particle size, 5 μm; Waters Associates) eluted at 1 ml/min with a 45-min gradient from 10 to 55% acetonitrile in water/0.1% trifluoroacetic acid, followed by a second 5-min gradient from 55 to 95%. UV absorbance was monitored at 215 nm, and 1-min fractions were collected for liquid scintillation counting. Conversion of $CHO(D_2)$-derived ^{3}H-PGE_2 to ^{3}H-PGB_2 was carried out as described (Stehle, 1982). In brief, radioactive HPLC fractions eluting with the retention time of standard PGE_2 (Sigma), obtained from incubations of $CHO(D_2)$ cells with the Ca^{2+} ionophore A23187 (4 μM) plus quinpirole (1 μM), were pooled and allowed to react with 0.5 M KOH in methanol/water (9:1 vol/vol) at room temperature for 15 min. After neutralization, the reaction mixture was dried under vacuum, and the residue was reconstituted in 0.1 ml of acetonitrile for HPLC analysis. HPLC was performed as described above, using a 60-min gradient from 20 to 40% acetonitrile in water/0.1% trifluoroacetic acid. Under these conditions, standard PGE_2 eluted at 22 min and PGB_2 at 33 min. In some experiments, radioimmunoassay (RIA) analysis was carried out on samples obtained from unlabeled $CHO(D_2)$ cells, either before or after HPLC fractionation. For RIA, we used an anti-PGE_2 antibody obtained from Sigma, following the manufacturer's instructions, and ^{3}H-PGE_2 from Amersham (192 Ci/mmol).

RESULTS AND DISCUSSION

$CHO(D_2)$ cells, labeled by incubation with [^{3}H]AA, were stimulated either with the Ca^{2+} ionophore A23187 (4 μM) or with A23187 plus quinpirole, a D_2 receptor agonist (1 μM). As previously described, the activation of D_2 receptors resulted in a marked enhancement of A23187-induced [^{3}H]AA release in the incubation medium (Felder et al., 1991; Kanterman et al., 1991; Piomelli et al., 1991). HPLC analysis of the medium revealed a major radioactive product released by A23187-stimulated $CHO(D_2)$ cells, which eluted at the retention time of authentic PGE_2 (Fig. 1a). On average, 360 ± 97 cpm per dish was recovered in the PGE_2 fraction after stimulation with A23187 alone and 1,040 ± 415 cpm per dish after stimulation with A23187 plus quinpirole (mean ± SEM, n = 5). No radioactive counts were detected at the retention time of PGE_2 in samples from control, unstimulated $CHO(D_2)$ cells.

The identity of the radioactive material eluting on HPLC as PGE_2 was confirmed by several methods. First, its formation was prevented by the cyclooxygenase inhibitor indomethacin (4 μM) (from 1,040 ± 415 to 28 ± 14 cpm per dish; n = 5; Fig. 1b). Next, when the radioactive fractions corresponding to this component were incubated with KOH/methanol, which converts selectively PGE_2 into PGB_2 (Stehle, 1982), HPLC analysis of the reaction mixture revealed the formation of a major labeled product at the

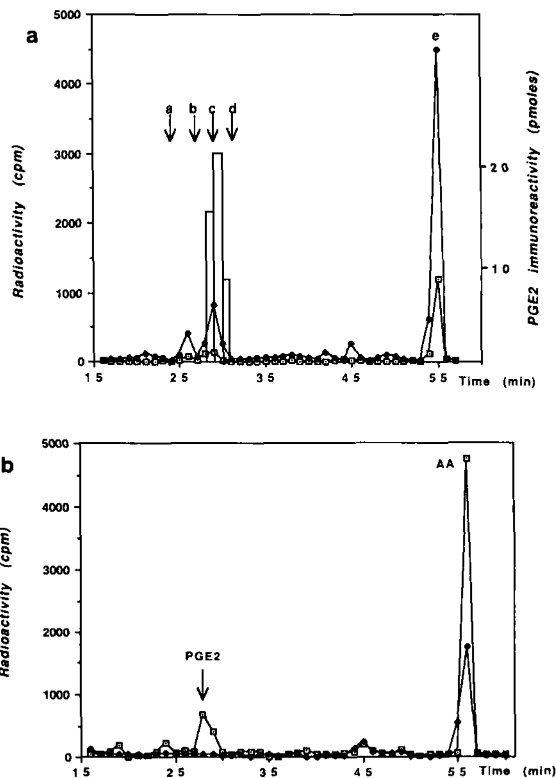

FIG. 1. Identification of PGE_2 produced by $CHO(D_2)$ cells. **a:** Analysis by HPLC and RIA. Cells, labeled by incubation with [^{3}H]AA, were stimulated for 30 min either with 4 μM A23187 (□), a Ca^{2+} ionophore, or with A23187 plus 1 μM quinpirole (♦), a D_2 receptor agonist. Samples of the incubation media were analyzed by reversed-phase HPLC, followed by liquid scintillation counting. Shown here is a tracing from one experiment, representative of five. In some experiments using unlabeled $CHO(D_2)$ cells, the fractions eluting from the HPLC column were dried under vacuum, and levels of i-PGE_2 were determined by RIA. Data, shown by the histogram, are from one experiment, with background subtracted. Arrows indicate the elution times of authentic prostanoid standards: a, 6-keto-$PGF_{1\alpha}$; b, $PGF_{2\alpha}$; c, PGE_2; d, PGD_2; and e, AA. **b:** Inhibition of PGE_2 formation by indomethacin, a cyclooxygenase inhibitor. Incubation of $CHO(D_2)$ cells was carried out in the presence of 4 μM A23187 and 1 μM quinpirole, without (□) or with (♦) 4 μM indomethacin. The tracing is representative of five experiments.

retention time of authentic PGB_2 (data not shown). Finally, analysis by RIA after HPLC fractionation showed a major component of PGE_2-like immunoreactivity at the retention time expected for this substance (Fig. 1a).

Two additional minor radioactive products, at retention times of 26 and 45 min, were seen occasionally on costimulation of CHO(D_2) cells with A23187 plus quinpirole. Formation of these metabolites was not inhibited by indomethacin, however, and their identity was not investigated further in this study. Thus, the results indicate that PGE_2 is a major metabolite of AA formed by A23187-stimulated CHO(D_2) cells and that its formation is augmented by the concomitant occupation of D_2 receptors.

When CHO(D_2) cells were stimulated with a combination of A23187 (4 μM), quinpirole (200 nM), and PGE_2, the resulting release of [^{3}H]AA was greater than with A23187 plus quinpirole alone. This effect was concentration dependent and half-maximal at a PGE_2 concentration of 100 nM (Fig. 2a). In contrast, the PG had no effect on either basal (not shown) or A23187-stimulated release (Fig. 2a). PGE_1, a cyclooxygenase metabolite of eicosatrienoic acid, was also effective in enhancing the D_2 response (EC_{50} = 140 nM; maximal stimulation, 221 ± 20% of 1 μM quinpirole effect; n = 3), whereas another PG, $PGF_{2\alpha}$, did not produce any significant increase at 1 μM (108 ± 14% of release produced by A23187 plus quinpirole; n = 4).

Together, the findings that PGE_2 represents a major AA metabolite in CHO(D_2) cells and that its application enhances D_2-mediated potentiation of [^{3}H]AA release suggest a possible participation of this PG in the D_2 response. The increased PGE_2 levels resulting from D_2 receptor stimulation may act to augment AA release further, by interacting with membrane receptors on the cell of origin or on neighboring cells.

One prediction of this hypothesis is that blockade of PGE_2 formation should result in a decreased overall liberation of [^{3}H]AA. To address this possibility, we studied the effects of indomethacin, a cyclooxygenase inhibitor, on the release of [^{3}H]AA induced either by A23187 alone or by A23187 plus quinpirole (0.5 μM) (Fig. 2b). To limit the possible loss of endogenously formed PGE_2, we carried out these experiments using an incubation medium of DMEM without BSA, which avidly binds hydrophobic molecules and is likely to reduce the concentration of free PGE_2. Under these conditions, quinpirole enhanced A23187-induced [^{3}H]AA release less potently than in the presence of BSA (by 180 instead of 400%) (Piomelli et al., 1991). This response was significantly reduced by treatment with indomethacin (Fig. 2b). The effect of indomethacin was unlikely to result from direct inhibition of phospholipase A_2 activity because the drug did not inhibit A23187-induced release at the concentration used here (4 μM) (Fig. 2b). Furthermore, inhibition by indomethacin could be overcome by the addition of exogenous PGE_2 (Fig. 2b). It is important that when incubations were carried out in DMEM plus BSA, indomethacin did not affect D_2-dependent potentiation of AA release (data not shown), suggesting that the contribution of PGE_2 to the D_2 response may be negligible under conditions where binding of the PG to BSA reduces its free concentration in the incubation medium.

Next, to test whether the amount of PGE_2 formed by stimulated CHO(D_2) cells may be sufficient to affect D_2 receptor-dependent AA response, we measured the levels of i-PGE_2 obtained in incubations with A23187 (4 μM) or with A23187 plus quinpirole (1 μM). In three experiments, we found that the average concentration of i-PGE_2 after a 30-min incubation of cells with A23187 was 10 ± 3 nM, and that after incubation with A23187 plus quinpirole it was 25.3 ± 7.1 nM. Inclusion of 4 μM indomethacin in the incubations reduced the formation of i-PGE_2 below control values (3.3 ± 1.1 nM). Thus, the levels of i-PGE_2 formed by stimulated CHO(D_2) cells are compatible with a role of this PG in modulating D_2 receptor-mediated potentiation of [^{3}H]AA release.

The mechanism underlying the ability of E-series PGs to enhance the D_2 AA response was not investigated in this study. In many tissues, including the brain, PGE_2 receptors of the EP_2 type are linked to the activation of adenylyl cyclase and to the augmentation of intracellular cAMP levels (Partington et al., 1980; Schaad et al., 1987; Weidenfeld et al., 1992). Likewise, PGE_2 and PGE_1 (which also binds to EP_2 receptors) raise intracellular cAMP levels in both wild-

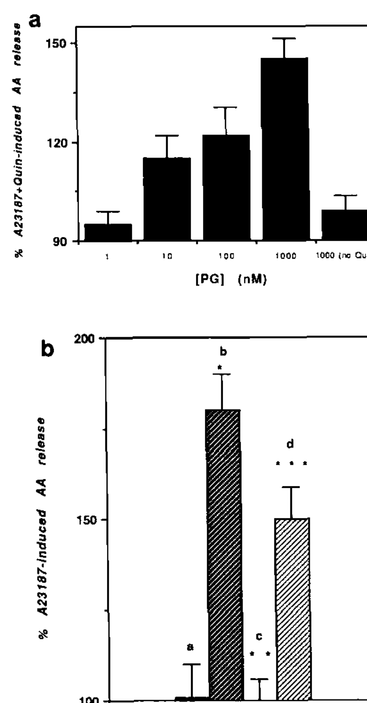

FIG. 2. a: Exogenous PGE_2 enhances release of [^{3}H]AA produced by application of the Ca^{2+} ionophore A23187 plus the D_2 receptor agonist quinpirole (Quin). CHO(D_2) cells (24-well plates), labeled by incubation with [^{3}H]AA, were incubated for 30 min in 1 ml of DMEM containing 4 μM A23187 plus 200 nM Quin and 1 nM–1 μM PGE_2. Samples (0.5 ml) of the incubation media were collected for counting. Results are expressed as percentages of the release induced by A23187 plus Quin (956 ± 144 cpm per well) and are mean ± SEM (bars) values from five experiments carried out in quadruplicate. The effects of all but 1 nM doses of PGE_2 were significantly different ($p < 0.05$) from both the effect of A23187 plus Quin and the effect of A23187 plus 1 μM PGE_2. **b:** Indomethacin (4 μM) prevents the potentiating effect of Quin on A23187-induced release, and exogenous PGE_2 restores it. Prelabeled CHO(D_2) cells were incubated in 0.75 ml of DMEM in the presence of A23187 (4 μM) plus indomethacin (4 μM) (column a), A23187 plus Quin (500 nM) (column b), A23187 plus Quin and indomethacin (column c), and the same plus PGE_2 (1 μM) (column d). Results are expressed as percentages of the release induced by A23187 and are mean ± SEM (bars) values from five experiments carried out in quadruplicate. *$p < 0.05$ versus A23187 only; **$p < 0.05$ versus A23187 plus Quin; ***$p < 0.05$ versus A23187 plus Quin and indomethacin.

type and transfected CHO fibroblasts. In $CHO(D_2)$ cells, 200 n*M* PGE_1 increased cAMP levels from 0.4 ± 0.06 to 1 ± 0.06 pmol per well (n = 3). Because the application of 8-bromo-cAMP, a membrane-permeable cAMP analog, enhances D_2 receptor-dependent potentiation of [^{3}H]AA release in these cells (Piomelli et al., 1991), it is possible that PGE_2 may act by stimulating adenylyl cyclase and by activating cAMP-dependent protein kinase. In agreement, we have recently shown that, in $CHO(D_2)$ cells cotransfected with either D_1 or β_2-adrenergic receptors, which like EP_2 are positively linked to adenylyl cyclase, stimulation of these receptors results in an enhanced D_2-dependent potentiation of AA release, which is accompanied by a rise in cAMP levels (Piomelli et al., 1991, 1992). It is unclear how the cell may integrate the costimulation of receptors, such as D_1/EP_2 and D_2, linked to opposing actions on adenylyl cyclase. A possible explanation is provided by a recent study suggesting that receptors coupled to "inhibitory" G_i proteins will cause elevation of cellular cAMP levels, provided that G_s is also active (Federman et al., 1992).

We found here that PGE_2 potentiates AA release induced by coapplication of A23187 plus quinpirole, but not release induced by A23187 alone. This suggests that PGE_2 may act by enhancing the functional coupling between D_2 receptors and the lipase responsible for release of AA, most likely a phospholipase A_2, rather than by modulating directly the activity of this enzyme. In agreement, the inhibitory effects produced by PGE_1 and cAMP on AA release and metabolism in other tissues are also thought to be produced through an indirect mechanism (Di Marzo et al., 1987, 1991).

Because the CNS has been shown to produce PGE_2 and to possess PGE_2 receptors positively linked to adenylyl cyclase (Malet et al., 1982; Yumoto et al., 1986), it will be interesting to test whether a modulation of dopaminergic transmission by this PG, similar to that described here for $CHO(D_2)$ cells, is exerted in brain areas such as the amygdala or the hypothalamus, where high densities of both PGE_2 and D_2 receptors are found (Malet et al., 1982; Watanabe et al., 1985; Bouthenet et al., 1991) and high levels of PGE_2 formation reported (Ogorochi et al., 1984).

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