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Dopamine D_2 receptor-induced COX-2-mediated production of prostaglandin E_2 in D_2-transfected Chinese hamster ovary cells without simultaneous administration of a Ca^{2+}-mobilizing agent

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Abstract

We have earlier demonstrated that dopamine stimulates the liberation of the prostaglandin E_2 (PGE_2) precursor, arachidonic acid, in Chinese hamster ovary cells transfected with the rat dopamine D_2 receptor (long isoform), also without concomitant administration of a Ca^{2+}-releasing agent [Nilsson *et al.*, Br J Pharmacol 1998;124:1651–8]. In the present report, we show that dopamine, under the same conditions, also induces a concentration-dependent increase in the production of PGE_2, with a maximal effect of 235% at ~100 μM, and with an EC_{50} of 794 nM. The effect was counteracted by the D_2 antagonist eticlopride, pertussis toxin, the inhibitor of intracellular Ca^{2+} release TMB-8, incubation in Ca^{2+}-free experimental medium, and PKC desensitization obtained by chronic pretreatment with the phorbol ester TPA. It was also antagonized by the non-specific cyclooxygenase (COX) inhibitor, indomethacin, and by the selective COX-2 inhibitor, NS-398, but not by the specific COX-1 inhibitor, valeryl salicylate. Both the non-specific phospholipase A_2 inhibitor, quinacrine, and an inhibitor of $cPLA_2$ and $iPLA_2$, AACOF3, counteracted the effect; in contrast, a selective $iPLA_2$ inhibitor, BEL, and a selective $sPLA_2$ inhibitor, TAPC, were ineffective. No effects of dopamine were obtained in control cells mock-transfected with the p3C vector only. The results reinforce previous assumptions that dopamine may interact with eicosanoid metabolism by means of D_2 receptor activation, and implicate an involvement of $cPLA_2$ and COX-2 in this effect. It is suggested that measurement of dopamine-induced PGE_2 production may serve as a convenient way to study D_2 receptor function *in vitro*.

Keywords: PGE_2; COX; Dopamine; Dopamine D_2 receptors; CHO cells

1. Introduction

PGE_2 is formed *via* the COX pathways from free arachidonic acid (AA) released from cell membrane lipids, and exert numerous physiological effects *via* prostanoid EP receptors (EPR) [1]. The two isoform-specific COX enzyme systems, constitutively expressed COX-1 [2], and inducible COX-2 [3], as well as EPR [4], are abundantly present in the central nervous system, and PGE_2 has been reported to influence neural transduction and signaling events in different ways [5–8]. In line with this, a possible involvement of PGE_2 in the pathophysiology of a number of neuropsychiatric disorders, including schizophrenia [9–11], Parkinson's [12] and Alzheimer's diseases [13] has been suggested.

We have earlier demonstrated that dopamine stimulates AA liberation in Chinese hamster ovary (CHO) cells

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Abbreviations: D_2 receptor, dopamine D_2 receptor; AA, arachidonic acid; PLA_2, phospholipase A_2; CHO, Chinese hamster ovary cells; CHO-D2 cells, CHO cells transfected with the D_2 receptor (long isoform); CHO-3C cells, CHO cells mock-transfected with the p3C transfection vector only; EBSS, Earle's balanced salt solution; α-MEM, modified Eagle's medium; PG, prostaglandin; PGE_2, prostaglandin E_2; EPR, prostanoid EP receptors; COX, cyclooxygenase; PTX, pertussis toxin; cAMP, adenosine 3′,5′-cyclic monophosphate; PKC, protein kinase C; A 23187, Ca^{2+} ionophore calcimycin; TMB-8, 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate hydrochloride; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; AACOF3, arachidonyltrifluoromethyl ketone; DTT, DL-dithiothreitol; TAPC, thioether amide-PC; BEL, bromoenol lactone; NS-398, *N*-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide; MAFP, methyl arachidonyl fluorophosphonate; EC_{50}, drug concentration causing half-maximal response.

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transfected with the rat D_2 receptor (long isoform), also without concomitant administration of a Ca^{2+}-releasing agent [14]. This finding contrasted the earlier notion that dopamine may potentiate AA release evoked by calcium-mobilizing compounds, but that presence of such agents is an indispensable prerequisite for dopamine to influence AA liberation [15–18].

Earlier reports have suggested that dopamine may enhance the effect of the Ca^{2+}-mobilizing ionophore A 23187 not only on AA release, but also on the formation of PGE_2 in D_2-transfected CHO cells [19,20]; no further characterization of this effect (e.g. in terms of potency or efficacy) has been provided. Given our previous finding that dopamine may elevate AA liberation, also without co-administration with a Ca^{2+} activator, we have now investigated the possible influence of dopamine *per se* on baseline PGE_2 synthesis in this transfection system. One purpose of these experiments was to shed further light on possible mechanisms underlying interactions between dopamine and eicosanoid metabolism in brain, and another one was to explore the feasibility of using PGE_2 formation as a parameter reflecting D_2 receptor function *in vitro*.

2. Materials and methods

2.1. Cell culture

CHO cells (CHO^{pro-} 5 strain, subclone CHO 10001 fibroblasts) expressing rat D_2 receptors (long isoform) due to transfection (approximately 500 fmol/mg protein, [^{3}H]spiperone binding) (CHO-L6 cells; in this paper referred to as CHO-D2 cells) were generously provided by Dr. R.M. Huff, Pharmacia & Upjohn [14,21–24]. For control experiments, CHO 10001 cells transfected with the p3C vector only (but with no dopamine receptor insert) (CHO-3C cells), and hence devoid of specific [^{3}H]spiperone binding, were used. The cells were kept frozen in liquid N_2 before being thawed and propagated, without ever reaching confluence, in 80-cm^2 flasks (Sarstedt) containing α-MEM with ribonucleosides and deoxyribonucleosides (Biochrom KG) + 10% charcoal/dextran-treated fetal bovine calf serum (HyClone) supplemented with penicillin G/streptomycin (PeSt) (100 unit/100 μg/mL) + L-glutamine (2 mM) (Biochrom). Incubation was undertaken at 37° in a water-saturated atmosphere of 5% CO_2 in air.

2.2. Experimental incubations

After splitting with trypsin–EDTA solution (Biochrom), stock grown cells were seeded at a density of 1.8×10^5 cells/mL into 24-multiwell plates (Sarstedt) (0.5 mL/well). The next day the subconfluent cells were washed (15 min twice) with Earle's balanced salt solution (EBSS) (Biochrom); thereafter the cells were incubated for 10 min with experimental drugs (incubation volume: 175 μL) in EBSS. Following experimental incubation, the medium was removed for subsequent assay.

2.3. PGE_2 measurement

PGE_2 levels in cell culture experimental medium aliquots were determined by the Prostaglandin E_2 Quantitative Competitive Enzyme Immunoassay (DE0100) (R&D Systems) in 96-well microplates read by a Spectramax 340 PC microplate spectrophotometer (Molecular Devices Corporation) using the SOFTmax PRO v. 3.0 software for Macintosh, in accordance with the manufacturer's protocol.

2.4. Drugs and chemicals

Dopamine HCl, eticlopride, pertussis toxin (PTX), TMB-8, TPA, and quinacrine were purchased from Sigma, and solubilized directly into EBSS or H_2O. TPA and BEL (Sigma) were solubilized in DMSO (Sigma; maximal final concentrations 0.03 and 0.1%, respectively). Valeryl salicylate and NS-398 were purchased from Cayman Chemical and solubilized in DMSO (maximal final concentrations 0.02 and 0.00001%, respectively). Indomethacin was solubilized in methanol (Merck; maximal final concentration 0.04%). AACOF3 was purchased from Biomol and solubilized in DMSO (maximal final concentration 0.05%). TAPC (Biomol) was solubilized in chloroform/methanol (1:1) (maximal final concentration 0.05%). Control groups were always given the same vehicle that was used for the corresponding treatment groups. Dopamine and the compound tested for its ability to counteract the effect of dopamine were always added simultaneously, with the exception of TPA and PTX; these two compounds were hence administered 22 hr before dopamine.

2.5. Data analysis

Since absolute PGE_2 levels always showed some interplate variability, PGE_2 levels were expressed as percentage of controls (given vehicle only) from the same plate before being used for further calculations. Data are given as means ± SEM of normalized PGE_2 values where N denotes number of independent determinations in each group as indicated in corresponding figure and table legends. Differences between groups were analyzed statistically using one-way analysis of variance (ANOVA) followed by Fisher's PLSD test. Percentage-normalized data in the concentration–response curve were analyzed according to the four-parameter logistic function: $E = [(\text{Min} - \text{Max})/(1 + \text{C}/\text{EC}_{50})^k)] + \text{Max}$, where Min = minimal response, Max = maximal response, C = concentration of dopamine, EC_{50} = dopamine concentration causing half-maximal response, and k = curve slope constant. The graph was generated

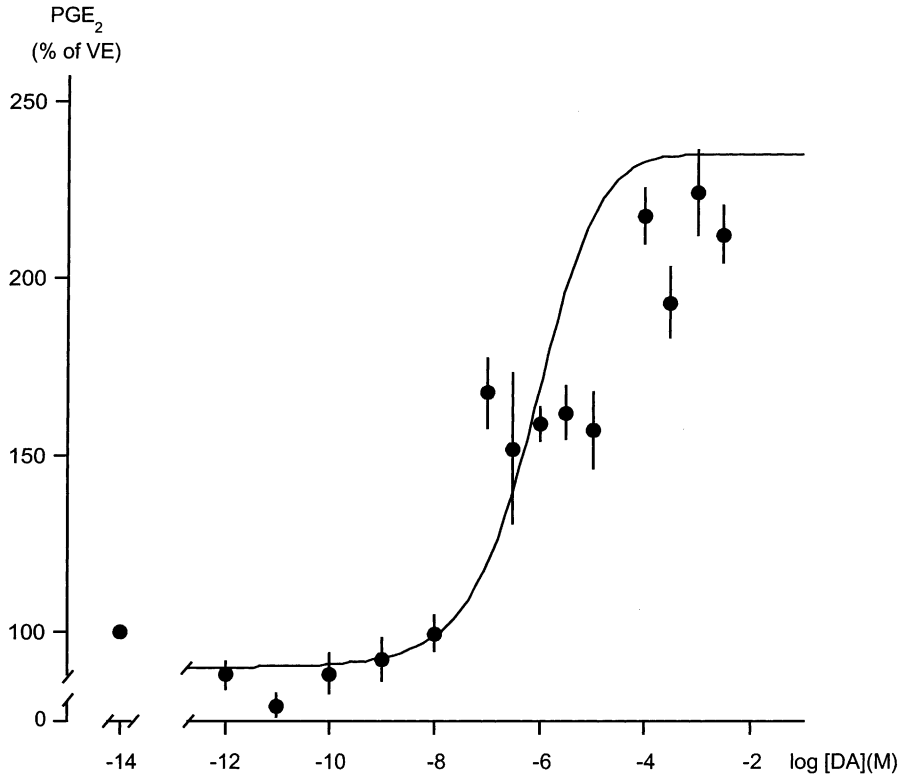

Fig. 1. Effects of various concentrations of dopamine (DA) on basal PGE_2 production in CHO-D2 cells. Solid curve represent fitted four-parameter logistic equation as described in Section 2. EC_{50} = 794 nM. Max effect = 235% of vehicles (VE) at ~100 μM. Points + bars represent mean ± SEM of values in a composite concentration–response curve from eight experiments, each performed in triplicate wells. Mean absolute PGE_2 concentration was 340 pg/mL in the group given vehicle only.

and parameters computed using the KaleidaGraph™ program (Synergy).

3. Results

3.1. Effects of dopamine on basal PGE_2 production in CHO-D2 cells

Dopamine, administered *per se*, produced a concentration-dependent increase in the production of PGE_2 in CHO-D2 cells with a maximal effect of 235% at ~100 μM, compared to vehicle-treated controls (100%). EC_{50} for dopamine was 794 nM (Fig. 1). Dopamine elicited no detectable effect on PGE_2 production in mock-transfected CHO-3C cells (not shown).

3.2. Effects of eticlopride on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was reduced by co-administration of eticlopride (Table 1). Eticlopride *per se* elicited an inhibitory effect on basal PGE_2 production when compared to vehicle-treated controls. This PGE_2-inhibiting effect of eticlopride was absent in mock-transfected CHO-3C cells expressing no D_2 receptors (not shown).

3.3. Effects of PTX on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production was abolished by pretreatment with PTX (−22 hr) (Table 1). PTX *per se* elicited an inhibitory effect on basal vehicle-treated controls.

3.4. Effects of Ca^{2+}-free experimental incubation on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was antagonized by experimental incubation in nominally Ca^{2+}-free salt solution (Table 1). Experimental incubation with Ca^{2+}-free salt solution *per se* elicited an inhibitory effect on basal PGE_2 production when compared to that of cells incubated in standard experimental salt solution containing Ca^{2+} (264 mg/L $CaCl_2$ dihydrate).

3.5. Effects of TMB-8 on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was antagonized by co-administration with TMB-8 (Table 1). TMB-8 *per se* elicited an inhibitory

Table 1
Effects of various treatments on dopamine-induced PGE_2 formation in CHO-D2 cells

Treatment	PGE_2 (% of vehicle)
Vehicle	100.0 ± 4.3
Dopamine (1 μM)	135.3 ± 4.4[a,b]
Eticlopride (1 μM)	76.7 ± 3.1[a,c]
Eticlopride + dopamine	91.8 ± 5.1
Vehicle	100.0 ± 2.6
Dopamine (1 μM)	129.4 ± 3.5[a,b]
PTX (200 ng/mL)	71.3 ± 2.7[a,c]
PTX + dopamine	59.5 ± 5.3
Vehicle	100.0 ± 3.5
Dopamine (3 μM)	157.5 ± 7.7[a,b]
Vehicle (−Ca)	71.2 ± 2.1[a]
Dopamine (−Ca)	89.0 ± 3.2
Vehicle	100.0 ± 6.2
Dopamine (3 μM)	180.0 ± 6.9[a,b]
TMB-8 (100 μM)	70.4 ± 2.2[a,c]
TMB-8 + dopamine	77.4 ± 3.7
Vehicle	100.0 ± 3.1
Dopamine (1 μM)	146.3 ± 7.8[a,b]
TPA (1 μM)	61.3 ± 4.6[a]
TPA + dopamine	62.2 ± 4.2

Data shown for each group represent means ± SEM, where N = 6 wells from a representative experiment that was replicated twice with similar results (N = 5 for the calcium-free incubation experiment). Mean absolute PGE_2 concentrations for vehicle groups were 312, 155, 301, 249, and 268 pg/mL for the eticlopride, PTX, calcium-free experimental incubation (−Ca), TMB-8, and TPA experiments, respectively. For details see Section 2.

[a] Significantly different vs. vehicle: $P < 0.001$.
[b] Significantly different vs. combination treatment: $P < 0.001$.
[c] Significantly different vs. combination treatment: $P < 0.05$.

effect on basal PGE_2 production when compared to vehicle-treated controls.

3.6. Effects of chronic TPA on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was antagonized by pretreatment with TPA (−22 hr) (Table 1). TPA *per se* elicited an inhibitory effect on basal PGE_2 production when compared to vehicle-treated controls.

3.7. Effects of quinacrine on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was antagonized by co-administration with quinacrine (Table 2).

3.8. Effects of AACOF3 on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was antagonized by co-administration with AACOF3 (Table 2).

Table 2
Effects of various PLA_2 inhibitors on dopamine-induced PGE_2 formation in CHO-D2 cells

Treatment	PGE_2 (% of vehicle)
Vehicle	100.0 ± 3.1
Dopamine(3 μM)	169.8 ± 12.9[a,b]
Quinacrine (50 μM)	86.6 ± 3.8
Quinacrine + dopamine	100.1 ± 3.1
Vehicle	100.0 ± 10.5
Dopamine (3 μM)	190.9 ± 14.8[a,c]
AACOF3 (10 μM)	82.8 ± 7.3[c]
AACOF3 + dopamine	134.4 ± 7.9
Vehicle	100.0 ± 2.8
Dopamine (3 μM)	165.2 ± 8.8[a]
BEL (10 μM)	99.7 ± 8.1[b]
BEL + dopamine	179.4 ± 12.6
Vehicle	100.0 ± 3.6
Dopamine (3 μM)	172.9 ± 14.8[a]
TAPC (10 μM)	87.3 ± 4.6[b]
TAPC + dopamine	171.2 ± 10.8

Data shown for each group represent means ± SEM, where N = 6 wells from a representative experiment that was replicated twice with similar results. Mean absolute PGE_2 concentrations for vehicle groups were 230, 137, 250, and 152 pg/mL for the quinacrine, AACOF3, BEL, and TAPC experiments, respectively. For details see Section 2.

[a] Significantly different vs. vehicle: $P < 0.001$.
[b] Significantly different vs. combination treatment: $P < 0.001$.
[c] Significantly different vs. combination treatment: $P < 0.01$.

3.9. Effects of BEL on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was not antagonized by co-administration with BEL (Table 2).

3.10. Effects of TAPC on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was not antagonized by co-administration with TAPC (Table 2).

3.11. Effects of indomethacin on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was antagonized by co-administration with indomethacin (Table 3). Indomethacin *per se* elicited an inhibitory effect on basal PGE_2 production when compared to vehicle-treated controls.

3.12. Effects of valeryl salicylate on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was not antagonized by co-administration with valeryl salicylate (Table 3).

Table 3
Effects of various COX inhibitors on dopamine-induced PGE_2 formation in CHO-D2 cells

Treatment	PGE_2 (% of vehicle)
Vehicle	100.0 ± 9.0
Dopamine (3 μM)	169.4 ± 14.9[a,b]
Indomethacine (4 μM)	57.2 ± 7.7[c]
Indomethacine + dopamine	63.9 ± 6.6
Vehicle	100.0 ± 2.0
Dopamine (3 μM)	141.2 ± 9.3[a]
Valeryl salicylate (100 μM)	92.8 ± 6.2[b]
Valeryl salicylate + dopamine	146.8 ± 9.5
Vehicle	100.0 ± 8.0
Dopamine (3 μM)	192.0 ± 22.6[a,b]
NS-398 (100 nM)	91.3 ± 6.0
NS-398 + dopamine	96.1 ± 2.6

Data shown for each group represent means ± SEM, where N = 6 wells from a representative experiment that was replicated twice with similar results. Mean absolute PGE_2 concentrations for vehicle groups were 78, 142, and 101 pg/mL for the indomethacine, valeryl salicylate, and NS-398 experiments, respectively. For details see Section 2.

[a] Significantly different vs. vehicle: $P < 0.001$.

[b] Significantly different vs. combination treatment: $P < 0.001$.

[c] Significantly different vs. vehicle: $P < 0.01$.

3.13. Effects of NS-398 on dopamine-stimulated PGE_2 production in CHO-D2 cells

The dopamine-induced increase in PGE_2 production in CHO-D2 cells was antagonized by co-administration with NS-398 (Table 3).

4. Discussion

This is the first report demonstrating that dopamine *per se*—without the concomitant administration of a Ca^{2+}-mobilizing agent—induces a concentration-dependent increase in basal PGE_2 production in D_2-transfected CHO cells. The observations that this effect could be counteracted by the specific D_2 antagonist, eticlopride, and is absent in mock-transfected CHO cells devoid of D_2 receptors, both indicate that it is mediated by D_2 receptors. Since the D_2 receptor is known to be coupled to G_i proteins, the finding that it was antagonized by pretreatment with PTX also is compatible with the notion that the effect is mediated *via* D_2 receptors.

We have previously shown that dopamine *per se* induces a concentration-dependent increase in the liberation of AA in D_2-transfected CHO cells. Supporting that the effect of dopamine on the production of PGE_2 is secondary to this increase in AA release, it was antagonized by quinacrine, a non-specific inhibitor of the PLA_2 enzyme responsible for AA liberation.

In order to clarify which of the different subtypes of this enzyme [25] that is involved in this effect, a number of selective inhibitors were also tested. In accordance with previous reports ruling out any role for the secretory form ($sPLA_2$) in CHO cells [26–29], the selective $sPLA_2$ inhibitor TAPC [30] did not counteract dopamine-induced PGE_2 formation; likewise, another $sPLA_2$ enzyme inhibitor, DTT [31], also was ineffective (data not shown). With respect to the other two main classes of PLA_2 enzymes, cytosolic PLA_2 ($cPLA_2$) and calcium-independent PLA_2 ($iPLA_2$), the 85-kDa $cPLA_2$ has generally been regarded as the most important PLA_2 subtype for AA liberation in CHO cells [32,33]; $iPLA_2$, however, has also been identified in CHO cells [34], and recently ascribed a major role in regulating phosphatidylcholine turnover [26]. The involvement of $cPLA_2$ and/or $iPLA_2$ in dopamine-induced increase in PGE_2 gained support from the finding that this effect was antagonized by a compound blocking both these enzymes, but having no effect on $sPLA_2$, AAFCO3 [35]. In conjunction with the finding that the selective $iPLA_2$ inhibitor, BEL [36], was devoid of effect, this observation suggests that dopamine-induced PGE_2 formation is probably mediated by $cPLA_2$, although a definite confirmation of this assumption cannot be obtained until selective $cPLA_2$ antagonists have been developed.

Apart from AAFCO3, another $cPLA_2$ and $iPLA_2$ inhibitor—MAFP—also was tested [37]; these data were, however, difficult to interpret, since the drug *per se* induced a marked increase in PGE_2 (data not shown). In line with this observation, Lin and Chen [38] recently reported that MAFP induces COX-2 gene expression as well as PGE_2 synthesis *in vitro*. The notion that COX-2 is involved in the formation of PGE_2 in CHO cells gained further support from the finding that the effect of dopamine was antagonized not only by the non-selective COX antagonist, indomethacin, but also by the COX-2 selective inhibitor, NS-398 [39]; in contrast, the COX-1 selective inhibitor valeryl salicylate [40] had no effect. Previous studies regarding the possible functional role of parental COX-1 and COX-2, respectively, in CHO cells are sparse. Experiments using CHO cells transfected with any of these enzymes, however, suggest that both COX-1 and COX-2 may influence PGE_2 production when overexpressed; one study suggesting COX-2 to be more effective in converting endogenously liberated AA to PGE_2, and COX-1 to be more important for the conversion of exogenously applied AA [41].

Further support for the interpretation that the effect of dopamine on PGE_2 production is mediated by the same pathways as the AA-relasing action described earlier is gained by the finding that it was abrogated by PKC desensitization obtained by long-term phorbol ester (TPA) pretreatment [14,16,17]. However, whereas this treatment was previously shown not to influence baseline AA release [14], it did cause a significant reduction not only in dopamine-induced PGE_2 production, but also in baseline PGE_2 levels. Tentatively, this observation could be explained by the fact that PKC is a strong regulator of COX expression [42].

That the dopamine-induced increase in PGE_2 production is calcium dependent is supported by the observations that

it was reduced by the inhibitor of intracellular Ca^{2+} release from reticular stores, TMB-8, and by incubation in balanced salt solution devoid of calcium ions; the observation that blockade of calcium-independent subtype of PLA_2 did not counteract the effect of dopamine, also is in line with this observation. Again these results are congruent with those previously reported regarding the influence of dopamine on AA release.

An important aspect of the results reported in this paper is that the clear-cut effects of dopamine on PGE_2 production in transfected CHO cells were observed without co-administration of a Ca^{2+}-mobilizing agent. This finding contrasts to several previous reports suggesting that simultaneous Ca^{2+} mobilization is an indispensable prerequisite for dopamine-induced increase in AA release in transfected CHO cells to occur [15–17]; moreover, in the only previous report suggesting an effect of dopamine on PGE_2 formation in transfected CHO cells, dopamine was given in conjunction with the Ca^{2+} mobilizer, A 23187 [19]. On the other hand, it is well in line with our previous report that dopamine does, in fact, influence AA mobilization *per se*, without the concomitant administration of Ca^{2+} activators [14].

The present observations that eticlopride caused a significant PGE_2-reducing effect when given *per se* to CHO-D2 cells, and that PTX reduced PGE_2 below baseline, are analogous to previous findings regarding the effect of D_2 antagonists and PTX on AA release in the same heterologous expression system reported earlier [14]. A tentative explanation to these results could be that the D_2 receptors in this system are characterized by a certain degree of precoupling in the absence of agonist, and that some D_2 antagonists may counteract this constitutive activity by means of inverse agonism [43–51]. Ongoing studies at our laboratory are aimed at further exploring this phenomenon.

The general notion of dopamine being capable of influencing the formation of prostaglandins is in line with several previous reports [12,52–55]. To what extent this effect of dopamine is of functional importance for the regulation of neurotransmission should be the subject of further studies, but different reports do suggest that certain effects of dopamine on, e.g. potassium channels [56,57], and on Na-K-ATPase activity [58], are indeed mediated by eicosanoids.

Of interest in this context are also *in vivo* studies in rodents, suggesting that COX inhibition stimulates D_2 agonist-induced circling behavior [59], and counteracts the catalepsy induced by D_2 antagonists [60–63]. Notably, intracerebroventricular administration of prostaglandins has been shown to elicit catalepsy qualitatively equivalent to that induced by D_2 antagonists such as haloperidol [62]. The possible involvement of prostaglandins in dopamine-related disorders such as schizophrenia and Parkinson's disease has been discussed in previous papers [9–12] and deserves further attention.

In vitro studies on the effect of dopamine receptor stimulation on various transduction systems in transfected cells have become an important approach for investigating interactions between receptors and G-proteins, and for evaluating how different compounds interact with the receptor studied. Regardless of the possible functional importance of the influence of dopamine on PGE_2 formation, we suggest that measuring PGE_2 levels after administration of D_2 ligands (without concomitant administration of a Ca^{2+} mobilizer) could be used as a convenient and informative strategy in this kind of investigations.

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