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Prostaglandin J_2 and Its Metabolites Promote Neurite Outgrowth Induced by Nerve Growth Factor in PC12 Cells

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Although A- and J-type prostaglandins (PG's) arrest the cell cycle at the G_1 phase *in vitro* and suppress tumor growth *in vivo,* their effects on neuronal cells have not so far been clarified. Here, we found promotion of neurite outgrowth as a novel biological function of PGJ's. In PC12h cells, PGJ's (PGJ_2, Δ^{12}-PGJ_2 and *15-deoxy*-$\Delta^{12,14}$-PGJ_2) promoted neurite outgrowth in the presence of nerve growth factor (NGF), whereas they themselves did not show such a promotion. The potency of promoting neurite outgrowth was PGJ_2 < Δ^{12}-PGJ_2 < *15-deoxy*-$\Delta^{12,14}$-PGJ_2. However, troglitazone, an activator of peroxisome proliferator-activated receptorγ (PPARγ), and other PG's including PGA_1, PGA_2 and PGD_2 did not promote neurite outgrowth. These results suggest that PGJ's promote neurite outgrowth independently of PPARγ activation. © 1999 Academic Press

Key Words: **prostaglandin J_2; prostaglandin D_2; peroxisome proliferator-activated receptor γ; neurite outgrowth; PC12 cells; nerve growth factor.**

Cyclopentenone PG's such as Δ^{12}-PGJ_2 and PGA_2 reportedly inhibit cell proliferation and induce various biological activities including anti-viral activity and osteogenesis (1, 2). These PG's have no cell-surface receptors but are actively transported into cells and accumulate in nuclei (3–5). Although their nuclear receptor remains unclear, they induce a variety of genes such as $p21^{WAF1/CIP}$ (6–8), p53 (9), *c-fos* (10), heme oxygenase-1 (11), HSP70 (12), IGF-1 (13), E-cadherin (14) and BIP/GRP78 (15). Recently, PGJ's (Fig. 1) were identified as natural ligands for PPARγ and found to promote adipocyte differentiation (16, 17). The potency of activating PPARγ is *15-deoxy*-$\Delta^{12,14}$-PGJ_2 > Δ^{12}-PGJ_2 > PGJ_2 ≫ PGD_2. These results indicate that PGJ's are not only anti-tumor agents but also play important physiological roles since PGD_2, a PGJ_2 precursor, is the most abundant PG in the CNS (18). PGD_2 is now well-known as a sleep inducer in mammalian brain (19), but the neuronal effects of PGJ's are not clear. Thus we addressed the question as to what biological function PGJ's possess in neuronal cells. Since PC12 cells serve as a useful model system for the study of neurite outgrowth (20), we used this system to test the neuronal function of PGJ's.

MATERIALS AND METHODS

Materials. NGF (2.5S) was purchased from Chemicon International. PGs (PGA_1, PGA_2, PGD_2, PGJ_2, Δ^{12}-PGJ_2 and *15-deoxy*-$\Delta^{12,14}$-PGJ_2) (Fig. 1) were obtained from Cayman Chemical. Troglitazone was a generous gift from Sankyo Co. These compounds were diluted in ethanol. The final concentration of ethanol was 0.1% and this concentration did not affect neurite outgrowth or cell survival.

Cell cultures. PC12h cells, a subline of PC12 cells, were maintained in 75 cm^2 flasks (Costar) containing 1:1 mixture of Dulbecco's Modified Eagle medium and F12 medium supplemented with 5% (v/v) of heat-inactivated (56°C, 30 min) horse serum (GIBCO) and 5% (v/v) of precolostrum newborn calf serum (Mitsubishi Kasei) (5/5 DF).

For evaluation of neurite outgrowth, the cells were transferred to collagen-coated 24 well plates (Costar) at a density of $1x10^4$ cells/cm^2 and incubated in 5/5 DF for 3 h. The medium was changed to serum-free DF, and the cells were incubated for 1 h, after which PG's were added. Thirty minutes later NGF (50 ng/ml) was added. The cells were then incubated for 24 h, and neurite (>10 μm)-bearing cells per total cells (around 70–100) in the same area were counted.

RESULTS

We examined the effects of PGJ's on neurite outgrowth induced by NGF in PC12h cells (Fig. 2). PC12h cells did not display any neurites when incubated in serum-free DF medium for 24 h (Fig. 2a). *15-deoxy*-$\Delta^{12,14}$-PGJ_2 alone did not have any significant promoting effect (Fig. 2b). Incubation of PC12h cells with NGF

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Abbreviations used: DF medium, 1:1 mixture of Dulbecco's Modified Eagle medium and F12 medium; NGF, nerve growth factor; PG, prostaglandin; PPARγ, peroxisome proliferator-activated receptor γ.

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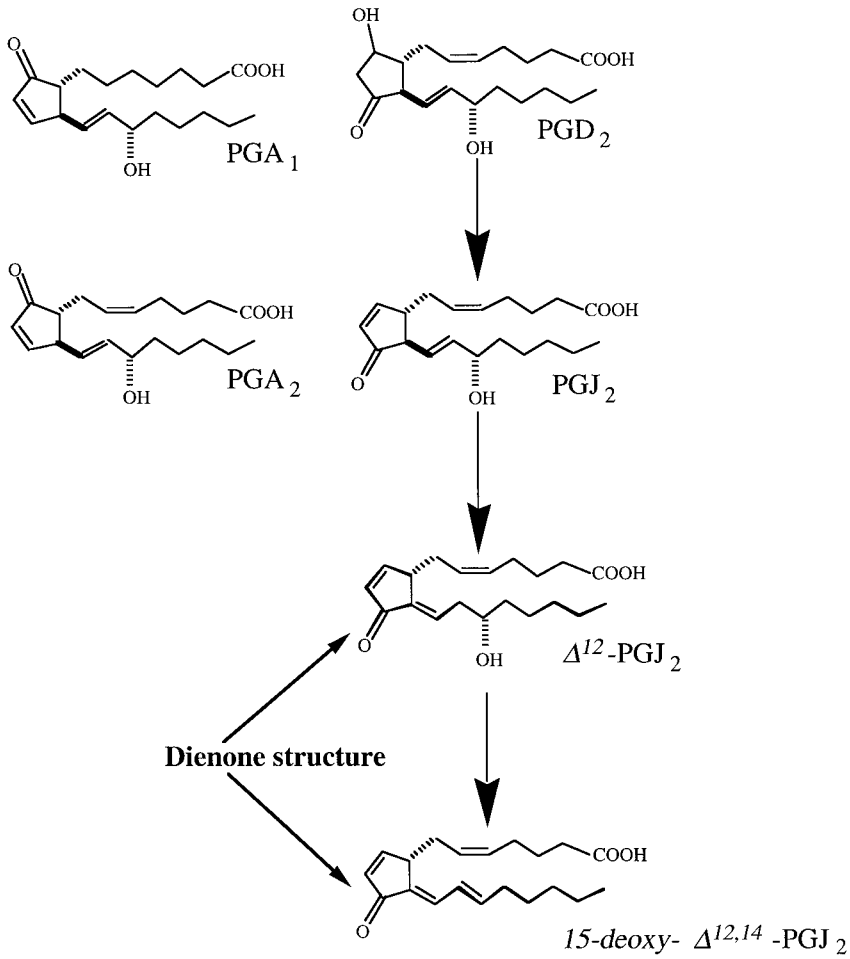

FIG. 1. Chemical structures of PG's used in the present study.

(50 ng/ml) for 24 hours induced neurite formation only slightly (Fig. 2c) since several days are required for neurite outgrowth in response to NGF. The presence of both *15-deoxy*-$\Delta^{12,14}$-PGJ_2 and NGF potently promoted neurite outgrowth (Fig. 2d). We also examined the effects of PGJ's, PGA's, PGD_2 and troglitazone, an activator of PPARγ (Fig. 3). Troglitazone, PGA_1, PGA_2 and PGD_2 did not promote neurite outgrowth at 0.1–5.0 μM, whereas PGJ_2, Δ^{12}-PGJ_2, and *15-deoxy*-$\Delta^{12,14}$-PGJ_2 did promote the outgrowth. The potency of promoting neurite outgrowth was *15-deoxy*-$\Delta^{12,14}$-PGJ_2 > Δ^{12}-PGJ_2 > PGJ_2 ≫ PGD_2. The promotion was observed within 48 hours, indicating that they only accelerated the speed of neurite outgrowth. Since higher concentrations (>0.5 μM *15-deoxy*-$\Delta^{12,14}$-PGJ_2; >2.0 μM Δ^{12}-PGJ_2 and PGJ_2) induced cell damage, we could not count the number of neurite-bearing cells in cultures containing these high concentrations of PG's.

DISCUSSION

The precursor of PGJ's, PGD_2, is the most abundant cyclooxygenase product in the rodent brain (18). PGD_2 is sequentially metabolized to PGJ_2, Δ^{12}-PGJ_2, and *15-deoxy*-$\Delta^{12,14}$-PGJ_2 (2, 16, 17). PGJ's promoted neurite outgrowth induced by NGF (50 ng/ml) in PC12h cells (Fig. 2). The potency of promoting neurite outgrowth was *15-deoxy*-$\Delta^{12,14}$-PGJ_2 > Δ^{12}-PGJ_2 > PGJ_2 ≫ PGD_2 (Fig. 3), indicating a gain in biological potency as the catabolism of PGD_2 proceeds. Since PGJ_2 is easily converted to Δ^{12}-PGJ_2, the activity of PGJ_2 may be mediated by Δ^{12}-PGJ_2. If it is the case, the dienone structure of *15-deoxy*-$\Delta^{12,14}$-PGJ_2 and Δ^{12}-PGJ_2 may be critical for promoting neurite outgrowth. The effective concentrations were 0.1–1.0 μM (Fig. 3), which were about ten times lower than those required for growth arrest and induction of various genes (6–15). Although the function of PGJ's in the brain is not clear yet, our results suggest that promoting neurite outgrowth is an intrinsic biological activity of PGJ's in neurons.

Forman et al. (16) and Kliewer et al. (17) reported that PGD_2 metabolites bind and activate PPARγ and promote adipocyte differentiation, indicating that they are critical regulators of the generation of adipocyte *in vivo.* The potency of activating PPARγ is *15-deoxy-*

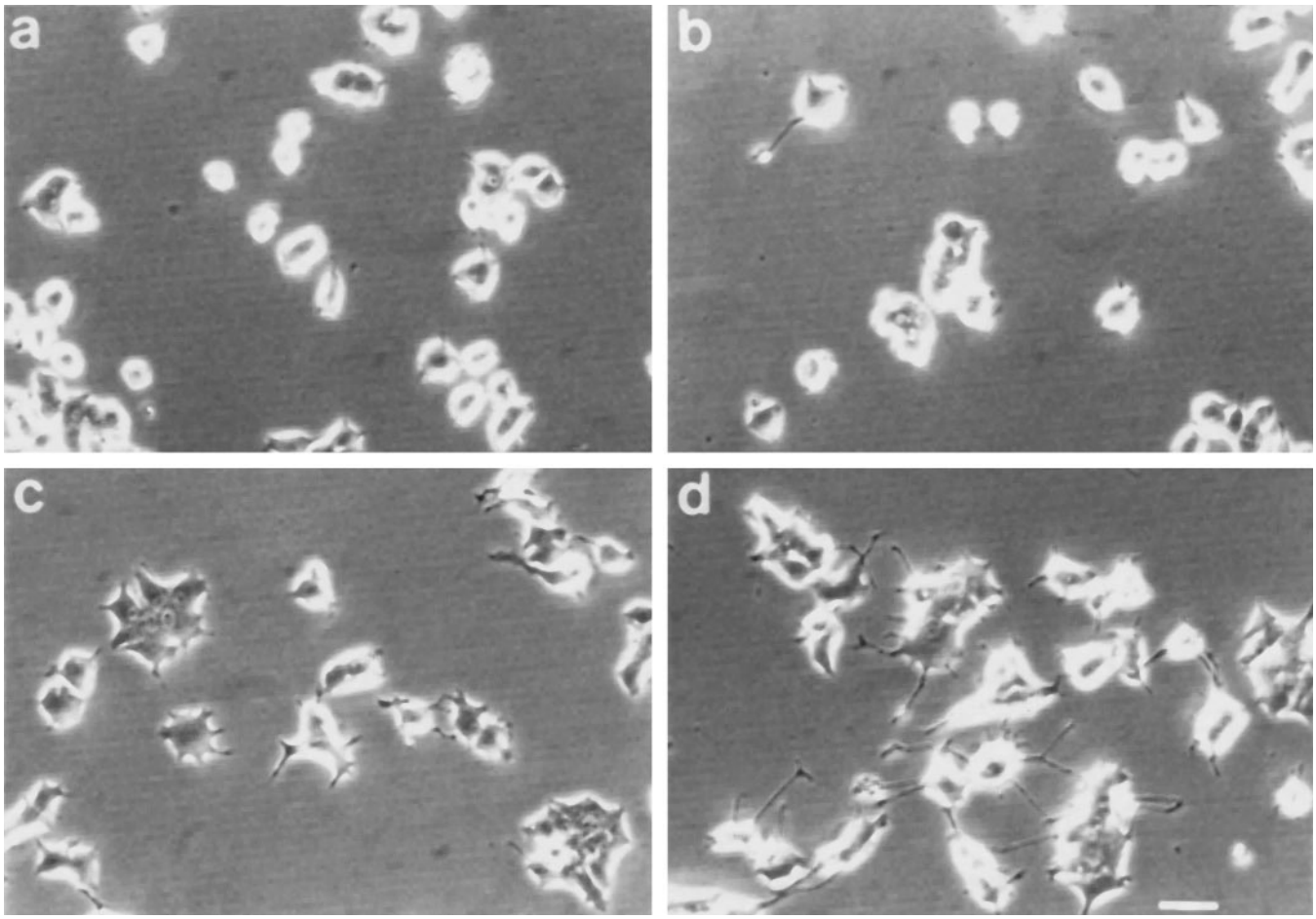

FIG. 2. Promotion of neurite outgrowth of PC12h cells induced by NGF and *15-deoxy*-$\Delta^{12,14}$-PGJ_2. PC12h cells were cultured in serum-free DF in the absence (a and b) or in the presence of NGF (50 ng/ml) (c and d) for 24 h. In (b) and (d), *15-deoxy*-$\Delta^{12,14}$-PGJ_2 (0.5 μM) was added 30 min before NGF challenge. The bar represents 10 μm.

$\Delta^{12,14}$-PGJ_2 > Δ^{12}-PGJ_2 > PGJ_2 ≫ PGD_2. This potency order is the same as that in the promotion of neurite outgrowth in the present study, suggesting that they induce the activity through PPARγ activation. However, the expression of PPARγ is highly specific to adipotissue (21), suggesting that PC12 cells do not express it. In addition, troglitazone, an activator of PPARγ, did not promote neurite outgrowth up to 10 μM (Fig. 3), indicating that PPARγ activation is not involved in the promotion of neurite outgrowth. It remains unclear yet as to what signaling pathway is required for the promotion of neurite outgrowth. PGJ's modulate expression of various genes, one of which may be essential for the promotion of neurite outgrowth in PC12 cells. One possible candidate is $p21^{WAF1/CIP1}$, because the overexpression of this gene reportedly accelerated neuronal differentiation in PC12 cells (22). Otherwise, PGJ's may activate MAP kinase activity, which is critical for neuronal differentiation in PC12h cells (23).

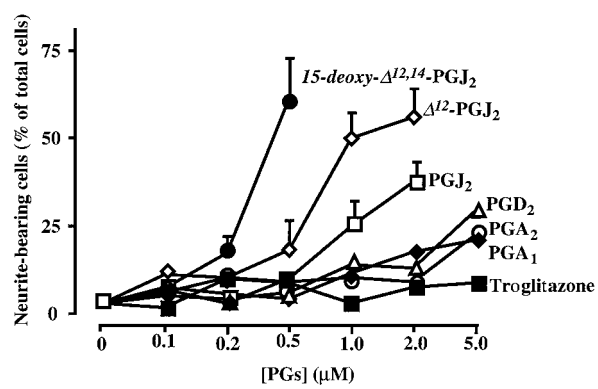

FIG. 3. Concentration-dependent promotion of neurite outgrowth in PC12h cells induced by PGJ's, PGA's and PGD_2. PC12h cells were cultured in the serum-free DF in the presence of NGF (50 ng/ml) with various concentrations of PG's for 24 h. Neurite (>10 μm)-bearing cells per total cells (around 70–100) in the same area were counted. The values are means ± S.D. (n = 4). Closed circles, *15-deoxy*-$\Delta^{12,14}$-PGJ_2; open diamonds, $\Delta^{12,14}$-PGJ_2; open squares, PGJ_2; open triangles, PGD_2; open circles, PGA_2; closed squares, troglitazone.

In conclusion, we found that PGJ's promoted neurite outgrowth induced by NGF in PC12 cells independently of PPARγ activation. This result suggests the possibility that PGJ's are critical regulators of neurite outgrowth or regeneration in the nervous system.

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