MAP Kinase Cascades Are Activated in Astrocytes and Preadipocytes by 15-Deoxy-Δ12-14-prostaglandin J2 and the Thiazolidinedione Ciglitazone through Peroxisome Proliferator Activator Receptor γ-independent Mechanisms Involving Reactive Oxygenated Species*

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15-Deoxy-Δ12-14-prostaglandin J2 (dPGJ2) and thiazolidinediones are known as ligands for the peroxisome proliferator activator receptor γ (PPARγ) a member of the nuclear receptor superfamily. Herein, we show that dPGJ2 activates, in cultured primary astrocytes, Erk, Jnk, p38 MAP kinase, and ASK1, a MAP kinase kinase kinase, which can be involved in the activation of Jnk and p38 MAP kinase. The activation kinetic is similar for the three MAP kinase. The activation of the MAP kinases is detectable around 0.5 h. The activation increases with dPGJ2 in a dose dependent manner (0–15 μM). A scavenger of reactive oxygenated species (ROS), N-acetylcysteine (NAC) at 20 mM, completely suppresses the activation of MAP kinases and ASK1, suggesting a role for oxidative stress in the activation mechanism. Other prostaglandin cyclopentenones than dPGJ2, A2, and to a lesser degree, A1, also stimulate the MAP kinases, although they do not bind to PPARγ. Ciglitazone (20 μM), a thiazolidinedione that mimics several effects of dPGJ2 in different cell types, also activates the three MAP kinase families and ASK1 in cultured astrocytes. However the activation is more rapid (it is detectable at 0.25 h) and more sustained (it is still strong after 4 h). NAC prevents the activation of the three MAP kinase families by ciglitazone. Another thiazolidinedione that binds to PPARγ, rosiglitazone, does not activate MAP kinases, indicating that the effect of ciglitazone on MAP kinases is independent of PPARγ. Ciglitazone and less strongly dPGJ2 activate Erk in undifferentiated cells of the adipocyte cell line 1B8. Ciglitazone also activates Jnk and p38 MAP kinase in these preadipocytes. Our findings suggest that a part of the biological effects of dPGJ2 and ciglitazone involve the activation of the three MAP kinase families probably through PPARγ-independent mechanisms involving ROS.

15-Deoxy-Δ12-14-prostaglandinJ2 (dPGJ2)† has been shown to bind and to activate peroxisome proliferator-activated recep-

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† The abbreviations used are: dPGJ2, 15-deoxy-Δ12-14-prostaglandin J2; ASK1, apoptosis signal-regulating kinase; Erk, extracellular signal-regulated kinase; IKK, IkB kinase; Jnk, c-Jun N-terminal kinase; NAC, N-acetylcysteine; MAP, mitogen-activated protein; MAPK, MAP kinase; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygenated species; FCS, fetal calf serum; DMEM, Dulbecco’s modified Eagle’s medium; DAPI, 4′,6-diamidino-2-phenylindole; PVDF, polyvinylidene difluoride.

‡ A. M. Lennon, M. Ramaugé, A. Dessouroux, and M. Pierre, unpublished results.
MAPK families are preferentially activated by stress and cytokines of the tumor necrosis factor α family. However, the specificity of activating stimuli is relative, depending on the cell type. For example, in astrocytes Erk is strongly activated by a number of stress factors including oxidative stress (11). We found that dPGJ2 and the thiazolidinedione cigitazone activate the MAP kinase cascades in astrocytes and in preadipocytes. We demonstrated that (i) ROS generation is required for this effect, (ii) the effect of dPGJ2 on MAP kinases is mimed by other prostaglandin cyclopentenones that do not bind to PPARγ, and (iii) the effect of cigitazone on MAP kinases was not mimed by an other thiazolidinedione ligand of PPARγ, rosiglitazone.

EXPERIMENTAL PROCEDURES

Materials

Sprague-Dawley rats were from our own breeding colony. Fetal calf serum (FCS), Dulbecco’s modified Eagle’s medium (DMEM), and Ham’s F-12 culture media were from Invitrogen. dPGJ2 was from Biomol and Cayman. Cigitazone, rosiglitazone, and prostaglandins A1 and A2 were from Cayman, N-Acetylcysteine (NAC) and 4,6-diamidino-2-phenylindole (DAPI) were from Sigma. Antibodies against active forms of thyronine and 20 nM insulin until complete differentiation into adipocytes. FCS content was reduced to 5% and supplemented with 1 nM triiodothyronine (T3) until confluence (preadipocytes). After cells reached confluence, they were further cultured for 3 days until the cells reached confluence at about 10 days. The cells were further cultured for several hours. The vehicle, 0.1% Me2SO, did not stimulate the MAP kinases. The MAP kinase activation was concentration dependent. Fig. 1B shows the activation of Erk, Jnk, and p38 MAP kinase after addition of increasing concentrations of dPGJ2 (0–15 μM) to cultured astrocytes for 2 h. For each MAP kinase family, the maximum activation was obtained at 15 μM dPGJ2.

For a number of stimuli (18–24), activation of MAP kinases requires the generation of reactive oxygenated species (ROS). Accumulation of ROS can be prevented by NAC, a scavenger of ROS. Cultured astrocytes were pretreated 15 min with 20 mM NAC before the addition of dPGJ2 (15 μM) to the culture medium. Fig. 1C shows that NAC completely prevents the activation of MAP kinases by dPGJ2, suggesting that the formation of ROS is involved. NAC does not affect the phosphorylation of MAP kinases in unstimulated cells (not shown).

Other Prostaglandin Cyclopentenones Activate MAP Kinase Cascades—dPGJ2 contains an α,β-unsaturated carbonyl group in the cyclopentane ring structure like prostaglandins A and other prostaglandins J. These prostaglandins are named prostaglandin cyclopentenones, and most of them share various biological effects (25), although several of them, like prostaglandins A, do not bind PPARγ. We tested whether prostaglandins A also activate MAP kinase cascades. Fig. 2 shows that both prostaglandins A1 and A2 are able to activate MAP kinases. However, they are weaker activators than dPGJ2. NAC also inhibits MAP kinase activation by prostaglandins A1 and A2.

MAP Kinase Activation by Cigitazone—As reported for other cells, the thiazolidinedione cigitazone promoted similar effects on astrocytes than dPGJ2. By example, cigitazone induced apoptosis like dPGJ2, although its effect was slower than that of dPGJ2 (Table 1). These observations prompted us to examine the effect of cigitazone on MAP kinase cascades.

Activation of Erk, Jnk, and p38 MAP kinase was observed after addition of 20 μM cigitazone to cultured astrocytes (Fig. 3A). The kinetic was similar for the three families of MAP kinases. Activation was detectable after 15 min and was sustained for several hours. The vehicle, 0.1% Me2SO, did not stimulate the MAP kinases as shown in Fig 1A. In this particular experiment, two forms of activated p38 MAP kinase were seen clearly, although in most of the experiments only one form was easily detectable. Also, in some experiments, an additional minor band was visible. We have not established the reason of these variations. The MAP kinase activation by cigitazone is concentration-dependent. Fig. 3B shows the activation of Erk, Jnk, and p38 MAP kinase after addition of increasing concentrations of cigitazone (0–20 μM) to cultured astrocytes for 2 h. For each MAP kinase family, the maximum activation was obtained with 20 μM cigitazone.

Cultured astrocytes were pretreated for 15 min with 20 mM NAC before the addition of cigitazone (20 μM) to the culture.

3 M. Pierre, unpublished observations.
medium. Fig. 3 shows that NAC reduces the activation of MAP kinases by ciglitazone.

**ASK1 Is Activated by dPGJ2 and Ciglitazone**—As we have shown previously (24), the MAP kinase kinase kinase ASK1, which can promote the activation of Jnk and p38 MAP kinase, is expressed in cultured astrocytes. The rate of migration on SDS-PAGE of ASK1 from stressed cells is decreased, which probably means that it is activated. Fig. 4 shows that ASK1 from cultured astrocytes treated with ciglitazone or dPGJ2 also shifts on SDS-PAGE. In astrocytes treated with dPGJ2, all the molecules of ASK1 clearly shifted. This shift promoted by dPGJ2 is prevented by NAC. In astrocytes treated by ciglitazone, only a fraction of ASK1 molecules shifted, and this was not clearly prevented by NAC. These observations show that ASK1 is probably activated in astrocytes by ciglitazone or dPGJ2, but that the mechanisms of activation may be different.

**Rosiglitazone, Another Ligand of PPARγ, Does Not Activate MAP Kinase Cascades**—The effect of rosiglitazone on MAP kinase cascades was studied and compared with that of ciglitazone. Astrocytes were incubated with various concentrations of rosiglitazone (2–20 μM) for 2 h. Astrocytes were also incubated with 20 μM rosiglitazone for various times (0.5–6 h). In all cases, no activation of MAP kinases was detected, although ciglitazone in the same cultures promoted a strong activation of MAP kinases. Fig. 5 illustrates the difference of the effects of ciglitazone and rosiglitazone on MAP kinase cascades.

**Ciglitazone, and to a Lesser Degree dPGJ2, Activate MAP Kinases in Preadipocytes**—Observations made with astrocytes prompted us to examine whether ciglitazone and dPGJ2 also activate the MAP kinase cascades in preadipocytes known to differentiate into adipocytes after treatment with ciglitazone or dPGJ2. Preadipocytes of the cell line 1B8, maintained in an undifferentiated state (see "Methods"), were incubated with ciglitazone (20 μM) or dPGJ2 (15 μM) for 2 h. The activation of MAP kinases was monitored by Western blot. Fig. 6A shows that ciglitazone strongly activated Erk but also p38 MAP kinase and Jnk. dPGJ2 less strongly activated Erk and did not stimulate Jnk and p38 MAP kinase. These observations suggest that MAP kinase activation, particularly Erk activation, could play a role in the action of ciglitazone and dPGJ2. Note that neither ciglitazone nor dPGJ2 activated MAP kinases in differentiated adipocytes (Fig. 6B).

**DISCUSSION**

We show that dPGJ2 and ciglitazone both promote the activation of MAP kinases (Erk, Jnk, and p38 MAP kinase) in
cultured astrocytes and preadipocytes. Ciglitazone and dPGJ2 are known as ligands of PPARγ/H9253, and their effects on MAP kinases might be mediated by this nuclear receptor. However, some works suggest that dPGJ2 and thiazolidinediones also may act by a nongenomic mechanism. By example Chawla et al. (26) have obtained PPARγ/H9253 deficient macrophages by using embryonic stem cells from mice deficient of PPARγ, and PPARγ ligands still exert anti-inflammatory effects in these macrophages. Our results indicate that the activation of MAP kinase cascades by dPGJ2 and ciglitazone in astrocytes probably involves PPARγ-independent mechanisms, because a classical ligand of PPARγ as rosiglitazone does not activate MAP kinases. Today, from the examination of the structures of these molecules (ciglitazone and rosiglitazone), we are not able to explain their different effect on MAP kinase activation. The observation that prostaglandins A2 and A1, which do not bind PPARγ but are prostaglandin cyclopentenones like dPGJ2, also activate MAP kinase cascades is also in favor of PPARγ-ide-
pemented mechanisms. Our results implicate that, to promote their biological effects, some thiazolidinediones act only through PPARγ-dependent mechanisms, while other thiazolidinediones act through PPARγ-dependent and -independent mechanisms. MAP kinases might be elements of the PPARγ-independent mechanisms.

Examination of the activation kinetic of MAP kinases shows that the effect of ciglitazone and dPGJ2 is rather rapid. Activation is easily detectable within 30 min after addition of dPGJ2 to the culture medium and after 15 min for ciglitazone. Maximum is reached after 2 h. Activation is more sustained with ciglitazone than with dPGJ2. This difference may be due to a different stability of these molecules in the culture medium, but other explanations are possible.

In previous work, we have shown that ASK1, a MAP kinase kinase kinase that activate Jnk and p38 MAP kinase cascades, is present in astrocytes and is activated by oxidative stress (24). In the present work, we show that ASK1 is activated by ciglitazone and dPGJ2, but that the activation of ASK1 by ciglitazone is not complete and is not blocked by NAC

An article by Takeda et al. (33), published while this manuscript was in preparation, shows that Erk cascade is activated in vascular smooth muscle cells by dPGJ2, pioglitazone, and troglitazone. This work and our work, taken together, show that the activation of MAP kinases by dPGJ2 and some thiazolidinediones can be obtained in various types of cells. The activation of the three MAP kinase families by dPGJ2 and some thiazolidinediones suggests a new concept of the molecular mechanism of action of these drugs. This may drive to use thiazolidinediones in therapeutic treatments for their ability to activate MAP kinases cascades or, contrary to this, to look for a reduction of undesirable side effects.

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