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Short Communication

INDUCERS OF ADIPOSE CONVERSION ACTIVATE TRANSCRIPTION PROMOTED BY A PEROXISOME PROLIFERATORS RESPONSE ELEMENT IN 3T3-L1 CELLS

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Abstract—Nonmetabolizable fatty acids are shown here to induce adipose conversion of 3T3-L1 preadipocytes as well as to activate transcription of a reporter plasmid promoted by a peroxisome proliferators response element. This dual activity was also observed using the peroxisome proliferator bezafibrate or the differentiation inducer isobutyl methylxanthine. The data suggest a role for a peroxisome proliferators activated receptor (PPAR) in adipose conversion induced by fatty acids, isobutyl methylxanthine, or xenobiotic amphipathic carboxylates.

Key words: fatty acids; peroxisome proliferators activated receptors; adipose conversion

Xenobiotic amphipathic carboxylates like bezafibrate or clofibrate have previously been shown by us to induce adipose conversion in primary epididymal preadipocytes or 3T3-L1 cells [1, 2]. Fatty acids have been similarly shown to activate the expression of marker genes for adipose conversion in ob17 and 3T3F44A cells [3–6]. Adipose conversion induced by amphipathic carboxylates is similar to that induced by IBMX.† However, the mode of action of the various inducers of adipose conversion still remains to be investigated.

In addition to adipose conversion, both xenobiotic amphipathic carboxylates as well as natural fatty acids induce peroxisome proliferation and peroxisomal β-oxidation activities in liver and other cells [7–12]. This inductive process has recently been found to be associated with transcriptional activation of the concerned peroxisomal genes mediated by a transduction pathway consisting of a PPAR and a PPRE in the promoters of the respective peroxisomal β-oxidation genes [13, 14].

A transduction pathway similar to that involved in peroxisomal proliferation induced by fatty acids and xenobiotic peroxisome proliferators may mediate as well adipose conversion induced by these agents. The present report addresses this question by analyzing the capacity of various inducers of adipose conversion as transcriptional activators of the expression of a CAT reporter plasmid promoted by peroxisomal AOX PPRE [18]. Natural fatty acids have been replaced here by nonmetabolizable fatty acyl analogues.

Materials and Methods

3T3-L1 preadipocytes were grown on plastic (Nunc) dishes at 37°C, under a 5% CO_2 humidified atmosphere, in DMEM medium (Beit Haemek, Israel) supplemented with 10% FCS (Gibco), 0.2 mg/mL streptomycin, and 200 U/mL penicillin. Differentiation was induced by adding the specified effectors and 0.1 μM dexamethasone to cells at confluence for 48–72 hours. The induction medium was then removed and replaced by a differentiation medium (i.e. growth medium supplemented with 4 mu/mL insulin). Differentiation was followed microscopically by detecting cells containing fat globules, and was quantitated by determining glycerol-3-P dehydrogenase activity, as previously described [1, 2].

Transfection was carried out by $(Ca)_3(PO_4)_2$-DNA coprecipitation [15] in cells grown to confluence on 6 cm dishes. Each dish, containing 5 mL medium, was transfected with 10 μg of the AOX-PPRE-CAT plasmid with or without 30 ng of an expression vector for PPAR. The cells were incubated in transfection medium for 6 hr at 37°C. The transfection medium was then removed, the cells were washed twice with PBS, and further incubated in induction medium containing 0.1 μM dexamethasone and effectors as specified. Seventy-two hours later, CAT activity was determined as described in ref. [16]. Cell extracts were prepared by four cycles of freeze-thaw and kept at −20°C until analyzed. Protein in extracts was determined by the method of Bradford [17].

The PPRE-CAT plasmid containing the 1.3 kb Δ −472/−129 5′-flanking sequence of the peroxisomal AOX gene linked to chloramphenicol acetyltransferase [18] was kindly donated by T. Osumi, Japan. The pSG5-PPAR expression vector [19] was kindly donated by S. Green, England. Dodecathioacetate (DTA) [20] was a gift from O. Spydevold, Norway. MEDICA 16 was synthesized as previously described [21].

Results and Discussion

We have previously shown that xenobiotic amphipathic carboxylates (e.g. clofibrate, bezafibrate) act as inducers of differentiation when added to confluent 3T3-L1 preadipocyte cultures for 48–72 hours at concentrations of 0.1–0.3 mM [2]. In contrast to the above-mentioned xenobiotics, adipose conversion could not be induced by natural fatty acids (oleic, palmitic, myristic, arachidonic) used at concentrations of 0.1–0.3 mM. At higher concentrations of fatty acids (0.5–1.2 mM), slight induction of adipose conversion could occasionally be detected, but mostly cell damage occurred. Adipose conversion of 3T3-L1 preadipocytes was, however, induced when nonmetabolizable fatty acids were used instead of the natural ones (Fig. 1). Thus, bromopalmitate, DTA, and MEDICA 16 added for 48 hr at concentrations of 0.15–0.3 mM were as potent as bezafibrate in

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† Abbreviations: IBMX, isobutyl methylxanthine; PPAR, peroxisome proliferators activated receptor; PPRE, peroxisome proliferators response element; AOX, acyl-CoA oxidase; CAT, chloramphenicol acetyl transferase; DTA, dodecathioacetate; MEDICA 16, β,β,β′,β′-tetramethylhexadecanedioic acid.

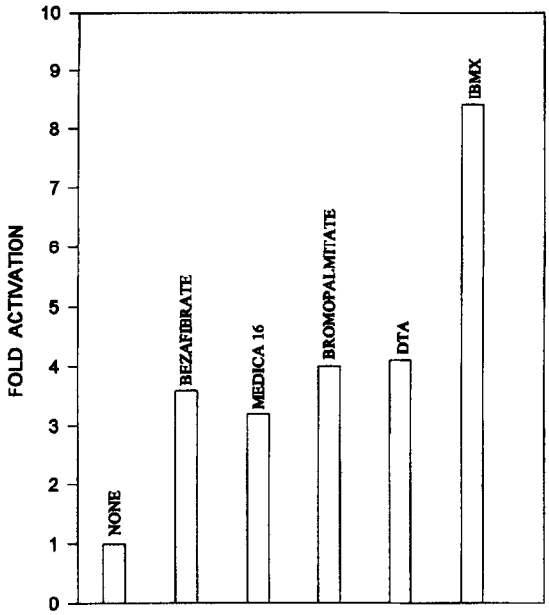

Fig. 1. Induction of adipose conversion in 3T3-L1 preadipocytes. Effectors (0.3 mM bezafibrate; 0.3 mM MEDICA 16; 0.15 mM bromopalmitate; 0.15 mM DTA; 0.5 mM IBMX) were added to confluent 3T3-L1 preadipocytes in induction medium as described in Methods. Glycerophosphate activity was determined three days after the removal of effectors. Each column represents the mean of two separate dishes differing by no more than 10%. One out of three independent experiments.

inducing differentiation. Higher concentrations often caused cell damage.

In addition to adipose conversion, these fatty acyl analogues also activated CAT transcription promoted by an AOX-PPRE (Fig. 2, empty bars). Activation of expression of the transfected reporter plasmid by nonmetabolizable fatty acids was as effective as that induced by bezafibrate, a well-established peroxisome proliferator. When cells were cotransfected with an expression vector for PPAR, CAT activity in the absence of added effectors was markedly increased and further increased in their presence (Fig. 2, shaded bars). Moreover, IBMX, a classical inducer of adipose conversion, was similarly effective in activating CAT expression provided that the cells were cotransfected with an expression vector for PPAR (Fig. 2). This effect of IBMX was not accounted for by its cAMP elevating activity, since but_2-cAMP did not promote CAT activity in the presence or absence of transfected PPAR (not shown). These transfection studies thus indicate that 3T3-L1 cells seem to possess both an endogenous receptor responding to various classes of inducers of adipose conversion and an endogenous ligand responding to an added transfected receptor.

Nonmetabolizable fatty acyl analogues, xenobiotic amphipathic carboxylates as well as IBMX, are shown here to act in 3T3-L1 preadipocytes both as inducers of adipose conversion and as activators of transcription promoted by PPRE. Hence, a transduction pathway consisting of PPAR and PPRE could mediate differentiation of preadipocytes by a variety of inducers of adipose conversion. This suggestion is supported by data reported by Chawla *et al.* [22] and Tontonoz *et al.* [23] showing that the PPAR gene is indeed expressed in adipocyte cell lines, and by Amri *et al.* [24], who cloned a fatty acid-activated PPAR receptor from ob-1771 cells. Direct proof for the claim that receptors of the PPAR family may play a central role in inducting adipose conversion has indeed been recently reported by Tontonoz *et al.* [25] showing that NIH-3T3 cells, originally incapable of adipose conversion, may be converted if stably transfected with $PPAR\gamma_2$.

IBMX activation of PPAR-mediated transcription of a PPRE-promoted reporter gene, as reported here, is worth noting because, in contrast to other xenobiotic or native PPAR activators, IBMX may not be defined as an amphipathic carboxylate. Still, it acts as an inducer of adipose conversion as well as an activator of the PPAR/PPRE transduction pathway. The IBMX effect could result from direct PPAR activation similar to that exerted by xenobiotic or native amphipathic carboxylates, or alternatively, could indirectly result from IBMX-induced increase in an endogenous PPAR activator. To distinguish between these two possibilities, PPAR-mediated activation of a PPRE-promoted reporter gene by IBMX was further verified in several cell lines. In addition to 3T3-L1 cells, activation by IBMX was observed in transfected NIH-3T3 nonconverting cells, thus indicating that activation of the PPAR/PPRE transduction pathway by IBMX in 3T3-L1 cells could not be secondarily ascribed to initiating the differentiation process by IBMX. However, no activation of PPAR/PPRE-mediated transcription by IBMX was observed in H_4 hepatoma cells or CV-1 cells, whereas xenobiotic amphipathic carboxylates served as PPAR activators in all four concerned cell lines. The cell-specific effect of IBMX may therefore indicate that adipose conversion and PPAR/PPRE activation by IBMX may be ascribed to an endogenous IBMX-induced PPAR activator. This difference between IBMX and the other xenobiotic carboxylate activators of PPAR is further corroborated by the specific requirement for transfected PPAR for activating transcription of the PPRE-promoted reporter gene by IBMX (Fig. 2). Nonmetabolizable xenobiotic carboxylates acting as direct PPAR activators may reach a higher cellular level as compared with that reached by an IBMX-induced endogenous activator, and may therefore activate even limiting endogenous PPAR that is available for binding to the transfected reporter plasmid. The identity of the putative endogenous PPAR activator induced by IBMX still remains to be investigated.

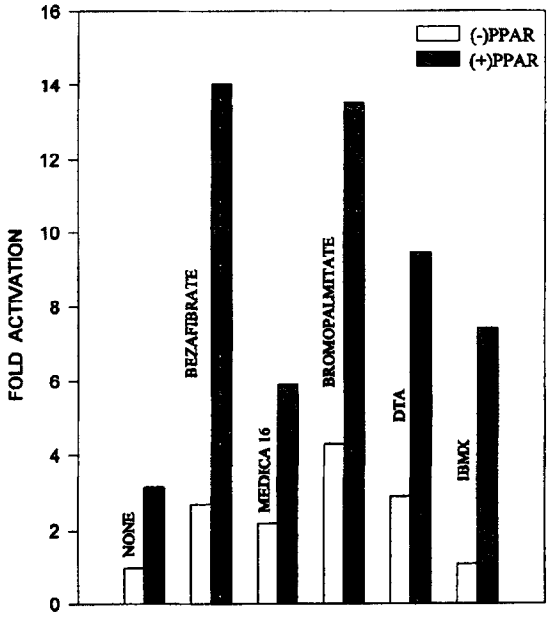

Fig. 2. Induction of PPRE-promoted CAT activity in 3T3-L1 preadipocytes. 3T3-L1 cells were transfected with a PPRE-promoted CAT reporter plasmid as described in Methods, and cotransfected (shaded bars) with an expression vector for PPAR. Following transfection, effectors were added to the induction medium as in Fig. 1, and CAT activity was measured 72 hours later. Each column represents the mean of two separate dishes differing by no more than 20%. One out of three independent experiments.

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