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SUMMARY OF DOCTORAL THESIS

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Title: **Specific action of prostacyclin on adipogenesis at different stages of cultured adipocytes**

（培養脂肪細胞の異なるステージでの脂肪細胞形成に対するプロスタサイクリンの特異的作用）

The present study attempted to clarify the specific action of prostacyclin, alternatively termed prostaglandin (PG) I_2, on adipogenesis at different stages of cultured adipocytes from two points of view. Initially, I studied the influence of pretreatment of cultured preadipocytes with arachidonic acid during the differentiation phase without a cAMP-elevating agent on adipogenesis after the maturation phase. Next research was conducted to unravel the effects of prostacyclin and selective agonists of prostanoid IP receptor on the storage of fats during the maturation phase of cultured adipocytes.

Arachidonic acid (AA), a member of n-6 polyunsaturated fatty acids, can be converted to several prostanoids with pro-adipogenic or anti-adipogenic effects through the arachidonate cyclooxygenase (COX) pathway with two types of COX isoforms, the rate-limiting enzymes of this pathway. Preadipogenic mouse 3T3-L1 cells have been utilized as a useful model cell culture system for studying different life stages of adipogenesis under the defined cultured conditions including growth, differentiation, and maturation phases. Recent studies have established that PGE_2 and $PGF_{2\alpha}$ are synthesized preferentially in cultured 3T3-L1 preadipocytes and serve as anti-adipogenic prostanoids. On the other hand, previous studies have reported the selective expression of lipocalin-type PGD synthase necessary for the biosynthesis of PGD_2 after the maturation phase of cultured 3T3-L1 cells. PGD_2 readily undergo the non-enzymatic dehydration to give biologically active PGJ_2 derivatives including 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) and Δ^{12}-PGJ_2. Of these, 15d-PGJ_2 is the most potent natural activator for the nuclear hormone receptor, peroxisome proliferator-activated receptor (PPAR)γ. We have also shown that cultured adipocytes after the maturation phase have the ability to increasingly produce endogenous PGs of J_2 series and contribute to the up-regulation of adipogenesis. Therefore, PGD_2 and the related PGJ_2 derivatives can be regarded as pro-adipogenic prostanoids. More recently, we have reported that endogenous synthesis of prostacyclin, PGI_2, is also positively regulated after the maturation phase of cultured 3T3-L1 adipocytes. Cultured 3T3-L1 preadipocytes have been usually exposed to the differentiation medium supplemented with 3-isobutyl-1-methylxanthine (IBMX), insulin, and dexamethasone to induce the program to drive the resting cells into adipocytes. The addition of exogenous AA to the differentiation medium has been shown to suppress the differentiation of cultured 3T3-L1 preadipocytes. On the other hand, earlier reports described that exogenous AA in the culture medium without IBMX was effective to induce the differentiation of Ob1771 preadipose cells and 3T3-F442A cells. Hence, we hypothesized that the opposite effects of exogenous AA on adipogenesis in different cell lines could be explained by presence or absence of IBMX, a cAMP elevating agent.

AA and the related prostanoids exert complex effects on the adipocyte differentiation depending on the culture conditions and life stages. Here, I investigated the effect of the pretreatment of cultured 3T3-L1 preadipocytes with exogenous AA during the differentiation phase without IBMX, a cAMP-elevating agent, on the storage of fats after the maturation phase. This pretreatment with AA stimulated appreciably adipogenesis after the maturation phase as evident with the up-regulated gene expression of adipogenic markers. The stimulatory effect of the pretreatment with AA was attenuated by the co-incubation with each of COX inhibitors. Among exogenous prostanoids

and related compounds, the pretreatment with MRE-269, a selective agonist of the IP receptor for PGI_2, strikingly stimulated the storage of fats in adipocytes. The gene expression analysis of arachidonate COX pathway revealed that the transcript levels of inducible COX-2, membrane-bound PGE synthase-1, and PGF synthase declined more greatly in cultured preadipocytes treated with AA. By contrast, the expression levels of COX-1, cytosolic PGE synthase, and PGI synthase remained constitutive. The treatment of cultured preadipocytes with AA resulted in the decreased synthesis of PGE_2 and $PGF_{2\alpha}$ serving as anti-adipogenic PGs although the biosynthesis of pro-adipogenic PGI_2 was up-regulated during the differentiation phase. Moreover, the gene expression levels of EP4 and FP, the respective prostanoid receptors for PGE_2 and $PGF_{2\alpha}$, were gradually suppressed by the supplementation with AA, whereas that of IP for PGI_2 remained relatively constant. Collectively, these results suggest the predominant role of endogenous PGI_2 in the stimulatory effect of the pretreatment of cultured preadipoccytes with AA during the differentiation phase without IBMX on adipogenesis after the maturation phase.

Earlier studies described that carbaprostacyclin, a stable analogue of PGI_2 also called prostacyclin, exerted pro-adipogenic effects on Ob1771 mouse-pre-adipose cells. Alternatively, growing 3T3-L1 cells have been reported to produce PGI_2 upon acute stimulation with calcium ionophore A23187. More recently we have provided the evidence that cultured 3T3-L1 adipocytes during the maturation phase are more capable of generating endogenous PGI_2 as determined by the amount of 6-keto-$PGF_{1\alpha}$ as the stable hydrolysis product of PGI_2 by its specific immunological assay, which is accompanied by the coordinated gene expression of PGI synthase and the prostanoid IP receptor for PGI_2. These findings lead us to suggest the pro-adipogenic action of endogenous PGI_2 as an autocrine factor in mature adipocytes. However, the action of natural PGI_2 appears to be uncertain due to the short half-life in biological fluids. By the extension of our recent results, we aim to obtain more insight into the role for prostacyclin and the related compounds in the up-regulation of adipogenesis in cultured adipocytes during the maturation phase. In this study, I tried to determine the specific action of the parent prostacylin and the related agonists or antagonists for the specific IP receptor on adipogenesis in combination with the agents that influence the activation of PPARγ and the elevation of cAMP. We discuss the mode of how prostacylin affects adipogenesis through the IP receptor during the maturation phase of adipocytes

My laboratory has previously shown that cultured adipocytes have the ability to biosynthesize PGI_2 called alternatively as prostacyclin during the maturation phase by the positive regulation of gene expression of PGI synthase and the prostanoid IP receptor. To clarify how prostacyclin regulates adipogenesis, I investigated the effects of prostacyclin and the specific agonists or antagonists for the IP receptor on the storage of fats during the maturation phase of cultured adipocytes. Exogenous PGI_2 and the related selective agonists for the IP receptor including MRE-269 and treprostinil rescued the storage of fats attenuated by aspirin, a COX inhibitor. On the other hand, selective antagonists for IP such as CAY10441 and CAY10449 were effective to suppress the accumulation of fats as GW9662, a specific antagonist for PPARγ. Thus, pro-adipogenic action of prostacyclin can be explained by the action mediated through the IP receptor expressed at the maturation stage of adipocytes. Cultured adipocytes incubated with each of PGI_2 and MRE-269 together with troglitazone, an activator for PPARγ, exhibited additively higher stimulation of fats storage than with either compound alone. The combined effect of MRE-269 and troglitazone was almost abolished by co-incubation with GW9662, but not with CAY10441. Increasing concentrations of troglitazone were found to reverse the inhibitory effect of CAY10441 in a dose-dependent manner while those of MRE-269 failed to rescue adipogenesis suppressed by GW9662, indicating the critical role of the PPARγ activation as a downstream factor for the stimulated adipogenesis through the IP receptor. Treatment of cultured adipocytes with cell permeable stable cAMP analogues or forskolin as a cAMP elevating agent partly restored the inhibitory effect of aspirin. However, excess levels of cAMP stimulated by forskolin attenuated adipogenesis. Supplementation with H-89, a cell permeable inhibitor for protein kinase A (PKA), had no effect on the promoting action of PGI_2 or MRE-269 along with aspirin on the storage of fats, suggesting that the promotion of adipogenesis mediated by the IP receptor does not require the PKA activity.