Published in final edited form as:

J Cell Physiol. 2014 April ; 229(4): 414–421. doi:10.1002/jcp.24473.

G Protein-Coupled Receptors and Adipogenesis: A Focus on Adenosine Receptors

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Abstract

G-protein coupled receptors (GPCRs) are a large family of proteins that coordinate extracellular signals to produce physiologic outcomes. Adenosine receptors (AR) are one class of GPCRs that have been shown to regulate functions as diverse as inflammation, blood flow, and cellular differentiation. Adenosine signals through four GPCRs that either inhibit (A1AR and A3AR) or activate (A2aAR and A2bAR) adenylyl cyclase. This review will focus on the role of GPCRs, and in particular, adenosine receptors, in adipogenesis. Preadipocytes differentiate to mature adipocytes as the adipose tissue expands to compensate for the consumption of excess nutrients. These newly generated adipocytes contribute to maintaining metabolic homeostasis. Understanding the key drivers of this differentiation process can aid the development of therapeutics to combat the growing obesity epidemic and associated metabolic consequences. Although much literature has covered the transcriptional events that culminate in the formation of an adipocyte, less focus has been on receptor-mediated extracellular signals that direct this process. This review will highlight GPCRs and their downstream messengers as significant players controlling adipocyte differentiation.

More than 30% of adults in the United States are obese (Flegal et al., 2010). This figure is particularly troubling given the medical sequelae of obesity, including coronary artery disease, hypertension, stroke and type II diabetes mellitus (Malnick and Knobler, 2006; Orpana et al., 2010). The need to understand the underlying molecular causes of increased adiposity are increasingly important. Knowledge of these processes will give us enhanced ability to prevent and treat obesity. An increase in body weight occurs when there is an excess of energy intake relative to energy output. While mild obesity is mainly a result of an enlargement in adipocyte size, more severe obesity involves an increase in the number of adipocytes through the differentiation of preadipocytes that reside within the fat pad (Rosen and MacDougald, 2006). Recruitment of preadipocytes and their differentiation to mature

cells is important for normal adipose tissue growth, remodeling and healthy expansion that is thought to help prevent the deleterious metabolic consequences of obesity. Much is known about the intracellular sequence of events that results in the differentiation of adipocytes, however, there has been less focus on the extracellular physiologic signals that regulate adipogenesis.

Nucleotides and their metabolites, like ATP and adenosine, signal to neighboring cells to regulate cellular processes such as tissue damage and repair and may play a role in cellular differentiation (Bours et al., 2006). ATP and adenosine are released from damaged cells during hypoxia, ischemia and inflammation (Linden, 2001; Chen et al., 2006; Fredholm, 2007; Eltzschig and Carmeliet, 2011). Extracellular ATP activates purinergic receptors or can be broken down to adenosine by ectoNTPDase, CD39, and ecto-5'-nucleotidase, CD73 (Zimmermann, 2000; Yegutkin, 2008). Adenosine acts on four adenosine receptors, a conserved group of G-protein coupled receptors (GPCRs), which are defined by their ability to inhibit (A1AR and A3AR) or stimulate (A2aAR and A2bAR) adenylyl cyclase (Jacobson and Gao, 2006; Fig. 1). Purinergic signaling is an important regulator of stem cell migration, proliferation, and differentiation (reviewed in Glaser et al., 2012).

This review will focus on the role of adenosine receptors and downstream signaling effectors in adipogenesis. We will begin with an overview of adipogenesis and the model systems used to study the process. We will review relevant literature linking G-protein coupled receptors, and more specifically adenosine receptors to adipocyte differentiation, and discuss the effect of two downstream effectors, cyclic AMP (cAMP) and calcium (Ca^{2+}), on adipocyte differentiation.

Adipogenesis in the Context of Adipose Tissue Remodeling

During the development of obesity, the adipose tissue expands by hypertrophy and by hyperplasia to accommodate excess nutrients (Rosen and MacDougald, 2006). It has been suggested that type II diabetes is a consequence of the inability of adipocytes to differentiate (Danforth, 2000; Jansson et al., 2003; Spalding et al., 2008). Adipogenesis occurs in response to excess nutrients in order to maintain metabolic homeostasis. The addition of adipocytes allows the organism to store more nutrients in the adipose tissue and prevents the pathologic accumulation of lipid in other organs like the liver, muscle, and heart. This redistribution of fat, or lipodystrophy, can lead to the development of type II diabetes. It is known that insulin sensitizers, like thiazolidinediones (TZDs), enhance de novo adipogenesis and hence increase adipose tissue storage capacity (Okuno et al., 1998; Chao et al., 2000). Furthermore, adipose tissue implantation into a diabetic lipodystrophic mouse model has been demonstrated to improve glucose tolerance (Gavrilova et al., 2000). The recruitment of new adipocytes improves insulin sensitivity as a result of an increase in storage capacity, but also due to an increase in insulin signaling by the newly generated adipocyte. Small, newly formed adipocytes are more insulin sensitive than their hypertrophied counterparts (Abbott and Foley, 1987; Tan and Vidal-Puig, 2008; Arner et al., 2010; Virtue and Vidal-Puig, 2010). Furthermore, large adipocytes have been associated with reduced insulin sensitivity and development of type II diabetes in humans (Weyer et al., 2000; Lundgren et al., 2007; Arner et al., 2010). Given the importance of adipocyte

turnover and recruitment in the pathophysiology of diabetes, understanding the control of adipocyte differentiation is clearly important.

Overview of Adipogenesis and Model Systems to Study this Process in Culture and In Vivo

Adipogenesis in culture

Adipogenesis has been extensively studied in vitro. The most frequently used preadipocyte cell lines are the 3T3-F442A and 3T3-L1 cells, which were isolated from the Swiss 3T3 cell line (Green and Meuth, 1974; Green and Kehinde, 1976). Another less commonly used cell line, Ob17 cells are derived from adipose precursors that were isolated from the epididymal fat pads of ob/ob adult mice (Negrel et al., 1978). Mesenchymal stem cells (MSCs), which can differentiate to several lineages including adipocytes, osteoblasts, and chondrocytes are commonly used primary cells for the study of adipogenesis, and can be isolated from the bone marrow and from umbilical cord blood (Caplan, 1991; Horwitz et al., 2005). Other primary cells that can be induced to differentiate to adipocytes in culture include mouse embryonic fibroblasts (MEFs) and preadipocytes found in the stromal-vascular fraction of mouse and human adipose tissue. To promote in vitro adipocyte differentiation, cells are grown to confluence and growth arrest. The addition of a cocktail of adipocyte-promoting factors causes the cells to re-enter the cell cycle and undergo several rounds of mitosis, known as mitotic clonal expansion (MCE), which appears to be a requirement for terminal adipocyte differentiation, at least in vitro (Otto and Lane, 2005). The cells enter a second, permanent period of growth arrest following the expression of certain key adipogenic factors (Umek et al., 1991; Altioek et al., 1997; Morrison and Farmer, 1999).

A defined set of transcription factors, including the ccaat enhancer binding proteins (C/EBP) family and peroxisome-proliferator activated receptor-gamma (PPAR γ) have been shown to be instrumental for adipogenesis in all model systems studied (Tontonoz et al., 1994; Barak et al., 1999; Rosen et al., 1999; Wu et al., 1999; Koutnikova et al., 2003). Early in differentiation, C/EBP β and C/EBP δ are transiently induced (Cao et al., 1991; Yeh et al., 1995; Darlington et al., 1998). The C/EBP family are basic-leucine zipper transcription factors, which act as hetero- or homodimers (Lekstrom-Himes and Xanthopoulos, 1998). Ectopic expression of C/EBP β is sufficient to induce differentiation of 3T3-L1 cells, while ectopic C/EBP δ expression only accelerates differentiation (Yeh et al., 1995). C/EBP β and C/EBP δ expression promotes the expression of PPAR γ , likely via C/EBP binding sites in the PPAR γ promoter (Zhu et al., 1995; Fajas et al., 1997). PPAR γ induces the expression of C/EBP α . C/EBP α overexpression in 3T3-L1 preadipocytes induces fat differentiation, while C/EBP α antisense RNA blocks differentiation (Lin and Lane, 1992; Freytag et al., 1994; Lin and Lane, 1994). PPAR γ and C/EBP α then promote the expression of genes necessary for adipocyte function, including glycerophosphate dehydrogenase, fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), glucose transporter type 4 (Glut4), insulin receptor, and fatty acid binding protein (FABP/aP2), and the accumulation of triglycerides (Spiegelman et al., 1993; Tontonoz et al., 1995a,b; Kubota et al., 1999; Rosen et al., 1999). PPAR γ has been described as the “master regulator” of adipocyte differentiation because the expression of PPAR γ is both necessary and sufficient for adipocyte differentiation (Tontonoz et al., 1994;

Rosen et al., 1999). Accordingly, the differentiation of preadipocyte cell lines is achieved with the use of various inducers, including insulin, glucocorticoids, methylisobutyl-xanthine (MIX) and TZDs (PPAR γ agonists). Insulin increases the percentage of differentiated cells and the amount of lipid accumulation (Girard et al., 1994). It is believed that the effect of insulin on differentiation occurs through cross-activation of the insulin growth factor-1 (IGF-1) receptor, as there are few insulin receptors on preadipocytes. The glucocorticoid, dexamethasone induces C/EBP δ expression and reduces the expression of preadipocyte factor-1 (pref-1), a negative regulator of adipogenesis (Wu et al., 1996; Smas et al., 1999). MIX, a phosphodiesterase inhibitor, enhances differentiation of 3T3-L1 cells, reportedly by increasing levels of adipocyte-promoting transcription factors (Cao et al., 1991).

Adipogenesis studied in vivo

Several studies have employed in vivo models to study the control of adipogenesis. It was reported that chimeric mice derived from ES cells with wild type PPAR γ and cells with a homozygous deletion of PPAR γ had no PPAR γ null cells in the white adipose tissue (Rosen et al., 1999). Furthermore, heterozygous deletion of PPAR γ leads to animals resistant to diet-induced obesity (Kubota et al., 1999). Homozygous deletion of C/EBP α results in decreased accumulation of fat in white and brown adipose tissue (Wang et al., 1995). On the other hand, C/EBP β and C/EBP δ are not necessary for adipocyte differentiation, as double knockout mice develop adipose tissue (albeit reduced compared to wild type mice) and express PPAR γ and C/EBP α (Tanaka et al., 1997). This suggests the existence of adipogenic transcriptional cascades that do not involve C/EBP β and C/EBP δ . Adipocyte determination and differentiation-dependent factor 1/sterol regulatory element binding protein 1 (ADD1/SREBP1), which was independently cloned as a factor from fat cells and as a liver protein that bound sterol-regulatory-elements in cholesterol regulatory genes, may be responsible for one of these pathways (Tontonoz et al., 1993; Brown and Goldstein, 1997). ADD/SREBP1 is induced during adipogenesis and increased by refeeding in mice, most likely due to increased insulin signaling (Kim and Spiegelman, 1996; Kim et al., 1998). Ectopic expression of SREBP1 in 3T3-L1 cells induces fat cell differentiation, while a dominant negative form of SREBP1 blocks differentiation in these same cells (Kim and Spiegelman, 1996; Fajas et al., 1999).

Recent research efforts have also focused on identifying and defining adipose precursors in vivo. Several attempts have been made to clarify the source of adipocytes (reviewed in Park et al., 2008 and Zeve et al., 2009). One group performed lineage tracing analysis using a PPAR γ reporter mouse and found that the progenitors resided in the adipose tissue vasculature, are present as early as post-natal day 1 (P1), and proliferate between P10 and P35 (as evidenced by BrdU staining; Tang et al., 2008). A second group identified progenitors within the adipose tissue and defined the cells by the presence of cell surface markers (lin⁻: CD29⁺:CD34⁺:Sca-1⁺:CD24⁺) and showed that they were capable of generating an adipose tissue depot in a lipodystrophic mouse model (Rodeheffer et al., 2008) and differentiating in response to high fat diet (Birsoy et al., 2008). More recently, the zinc finger protein, Zfp423 was shown to play a role in preadipocyte determination (Gupta et al., 2010). The group that identified this factor generated a mouse model in which Zfp423 drives GFP expression (Gupta et al., 2012). This mouse model will provide an excellent tool to

facilitate the isolation and study of a more purified population of preadipocytes from adipose tissue.

G-Protein Coupled Receptors and Accessory Proteins and Adipogenesis

The P2Y purinergic receptors are a class of G-protein coupled receptors that may play a role in adipogenesis (Ralevic and Burnstock, 1998; Abbracchio et al., 2006; Burnstock, 2007). Upon binding P2Y receptors, ATP enhances the effect of adipogenic hormones on the differentiation of 3T3-L1 cells by altering the organization of actin filaments and promoting conversion to a lipid-laden cell (Omatsu-Kanbe et al., 2006). ATP also inhibits the proliferation and stimulates the migration of MSCs (Ferrari et al., 2011). Acting through P2Y1 and P2Y4 receptors, ATP was also shown to increase adipogenesis of human bone marrow-derived MSCs by promoting lipid accumulation and the expression of PPAR γ (Ciciarello et al., 2013).

Short chain fatty acids acetate and propionate are ligands for G-protein coupled receptor 43 (GPCR43), while medium and long chain free fatty acids are ligands for G-protein receptor 120 (GPR120) (Hirasawa et al., 2005; Hong et al., 2005). GPCR43 couples to G α_i and GPR120 couples to G α_q (Brown et al., 2003; Hirasawa et al., 2005). GPCR43 and GPR120 expression is high in mouse adipose tissue and in isolated adipocytes but not in preadipocytes in the stromal-vascular fraction (Hong et al., 2005; Gotoh et al., 2007). Adipose tissue GPCR43 and GPR120 is upregulated in mice fed high fat diet as compared to standard diet and GPCR43 and GPR120 expression increases during the differentiation of 3T3-L1 cells (Hong et al., 2005; Gotoh et al., 2007). Knockdown of GPCR43 or GPR120 expression by siRNA decreases adipocyte differentiation (Hong et al., 2005; Gotoh et al., 2007). GPR120 KO mice on a high fat diet have increased weight gain, glucose intolerance, and hepatic lipid deposition as well as decreased adipocyte differentiation (Ichimura et al., 2012). Moreover, GPR120 expression in human adipose tissue is greater in obese individuals as compared to lean individuals and a loss of function mutation in GPR120 is associated with an increased risk of obesity in a European population (Ichimura et al., 2012). However, there is no relationship between GPCR43 and adipogenic potential of preadipocytes isolated from human omental adipose tissue (Dewulf et al., 2013).

G-protein receptor 103 (GPR103) is a receptor whose ligand is QRFP, an orexigenic neuropeptide made up of 43 amino acids residues, and couples to G α_q to enhance intracellular Ca²⁺ levels (Fukusumi et al., 2003; Jiang et al., 2003; Takayasu et al., 2006). Although predominantly expressed in the nuclei of the hypothalamus, one subtype of the receptor, GPR103b, is found in multiple tissues including adipose tissue and similarly QRFP peptides are mostly produced in the brain but are also found in adipose tissue (Fukusumi et al., 2006; Zhang et al., 2007). QRFP is a member of the RFamide family of peptides which all contain carboxy-terminal arginine (R) and amidated phenylalanine (F) residues (Price and Greenberg, 1977). The RFamide family has been associated with regulation of adipocyte differentiation (Fuku-sumi et al., 2006; Zhang et al., 2007). The expression of GPR103b increases with differentiation of 3T3-L1 cells (Mulumba et al., 2010). QRFP peptides increase adipocyte differentiation in 3T3-L1 cells (Mulumba et al., 2010). Moreover, the expression of GPR103b increases in the epididymal adipose tissue and

isolated epididymal adipocytes of mice fed a high fat diet as compared to normal diet (Mulumba et al., 2010).

β -Adrenergic receptors are coupled to G α s proteins. The expression of β 2 adrenergic receptor (β 2-AR) and β 3-AR increases during the differentiation of mouse bone marrow-derived MSCs (Li et al., 2010). The β -agonist, isoproterenol, inhibits adipocyte differentiation by activating β 2-ARs and β 3-ARs but not β 1-ARs (Li et al., 2010). Furthermore, the inhibitory action of the β -adrenergic receptors is due to activation of PKA (Li et al., 2010). The thyroid stimulating hormone (TSH) receptor is another G-protein coupled receptor whose ligand is a hormone and couples to G α s. Transfection of a constitutively active TSH receptor leads to a decrease in adipogenesis (Zhang et al., 2009). However, adipogenesis was also shown to be enhanced by TSH in cultured embryonic stem cells (Lu and Lin, 2008).

G-Protein coupled receptor kinases (GRKs) reduce GPCR signaling by phosphorylating serine/threonine residues of G-protein-coupled receptors and promoting the binding of arrestins and subsequent receptor internalization (Kohout and Lefkowitz, 2003; Lefkowitz, 2007). GRK5 is highly expressed in white adipose tissue (Wang et al., 2012). GRK5 KO mice fed a high fat diet gain less weight and have less adipose tissue mass as compared to control mice despite no differences in feeding or energy expenditure (Wang et al., 2012). GRK5 KO mice have reduced expression of lipogenic and adipogenic genes in WAT as compared to control mice (Wang et al., 2012). Furthermore, MEFs and stromal-vascular cells from GRK5 KO mice have impaired adipogenesis during in vitro adipogenic induction (Wang et al., 2012).

Adenosine Receptors and Adipogenesis

Adenosine is continuously released from fat cells in vitro and subcutaneous adipose tissue in vivo (Schwabe et al., 1973; Fredholm, 1976). The release of adenosine is increased by ischemia, catecholamine exposure, or sympathetic nerve stimulation (Fredholm and Sollevi, 1981). The A2bAR and A2aAR are predominantly expressed on preadipocytes, while mature adipocytes mainly express A1AR (Vassaux et al., 1993; Borglum et al., 1996). During adipogenesis the expression of the adenylyl cyclase stimulatory adenosine receptors decreases and the expression of the adenylyl cyclase inhibitory adenosine receptors increases (Ravid and Lowenstein, 1988; Vassaux et al., 1993; Borglum et al., 1996; Gharibi et al., 2011). The A2bAR has been shown to be the functionally dominant adenosine receptor in MSCs, while the A2aAR has been shown to be important for the proliferation and differentiation of murine bone marrow-derived MSCs into adipocytes (Evans et al., 2006; Katebi et al., 2009; Gharibi et al., 2011). In Ob1771 preadipocytes, 5'-N-Ethylcarboxamidoadenosine (NECA), a non-specific adenosine receptor agonist, was shown to increase cAMP, but CGS 21680, an A2aAR specific agonist, did not increase cAMP, suggesting that the A2bAR is the predominant adenosine receptor in preadipocytes (Borglum et al., 1996).

Most published studies on the role of adenosine receptors in adipogenesis have examined the effect of the receptors with pharmacologic agonists and overexpression studies in cell lines. An early study showed that NECA treatment of rat primary preadipocytes increases the

number of differentiated cells (Vassaux et al., 1993). A more recent study showed that activation of the A2aAR and A1AR in MSCs enhances adipocyte differentiation and lipid accumulation. (Gharibi et al., 2011) It was reported that A2aAR expression promoted PPAR γ and C/EBP- α expression, while A1AR increased lipogenesis and lipid accumulation, rather than differentiation (Gharibi et al., 2011). The A1AR has also been shown to play a role in other functions of the adipocyte, including blocking lipolysis, promoting leptin secretion, and protecting against insulin resistance (Fredholm, 1978; Cheng et al., 2000; Rice et al., 2000; Dong et al., 2001; Schoelch et al., 2004; Johansson et al., 2007; Szkudelska et al., 2009). Transfection of A1AR into a murine osteoblast precursor cell line, 7F2, or into ob17 cells increased adipocyte marker expression and lipid accumulation (Tatsis-Kotsidis and Erlanger, 1999a,b; Gharibi et al., 2012). Transfection of the A2bAR in these cells also augmented osteoblast mRNA expression and inhibited adipogenesis, even in the presence of adipocyte differentiation media (Gharibi et al., 2012).

The mechanism underlying the effect of the adenosine receptors on adipocyte differentiation has not been elucidated. The adenosine receptors couple to G α s (A2aAR and A2bAR), G α i (A1AR and A3AR) and to G α q (A2bAR) and all the adenosine receptors can activate mitogen-activated protein kinase (MAPK) signaling. We will next explore the role of these signaling pathways on adipogenesis.

cAMP and Adipogenesis

G-protein coupled receptors that are bound to G α s activate adenylyl cyclase and increase cAMP levels. The role of cAMP in adipogenesis is debated. Early studies suggested that cAMP may promote adipogenesis. MIX, a phosphodiesterase inhibitor, is often used in vitro to stimulate adipocyte differentiation, most likely by increasing the level of C/EBP β (Cao et al., 1991). MIX treatment throughout differentiation marginally increased adipocyte differentiation in rat primary preadipocytes cells (Bjorntorp et al., 1980) while MIX or forskolin treatment for the first two days following induction of differentiation increased differentiation in these same cells (Wiederer and Loffler, 1987). Treatment of 3T3-L1 cells with dibutyryl-cAMP (a cAMP analog), forskolin, theophylline, or MIX for 48 h increased adipogenesis (Brandes et al., 1990; Hauner, 1990; Schmidt et al., 1990). Similarly, a more recent study found that the addition of forskolin (100 μ M), MIX, or Sp-cAMP (a cAMP analog) enhanced the adipocyte differentiation of human bone-marrow derived MSCs (Yang et al., 2008). However, other studies have suggested that cAMP does not enhance adipocyte differentiation. For example, neither forskolin, 8-Br-cAMP nor MIX treatment of preadipocytes from porcine adipose tissue enhanced adipogenesis when applied 1-7 days prior to induction (Boone et al., 1999). It appears that the level and timing of cAMP elevation during differentiation may be the most important factor in predicting the effect on adipogenesis. Yarwood et al. found that 3T3-F442A cells treated for 3 days with agents that increase cAMP levels, such as 50 μ M forskolin, cholera toxin or CPT-cAMP inhibited adipogenesis. In contrast, MIX promoted adipogenesis in these same cells. The authors then tested various concentration of forskolin on adipocyte differentiation and found that low levels of forskolin (1 nM) enhanced adipogenesis, while higher levels of forskolin (above 1 μ M) inhibited differentiation (Yarwood et al., 1995). The timing of cAMP elevation may also change the effect on adipocyte differentiation. For example, one study found that a 2-

day treatment of 3T3-L1 cells with the cAMP analog, 8-Br-cAMP, or forskolin could replace MIX in the adipogenic induction cocktail, but 15 min pretreatment with forskolin (1 μ M) or 8-Br cAMP significantly reduced adipogenesis in the presence of MIX (Li et al., 2008). The authors also showed that treatment with an adenylate cyclase inhibitor 15 min prior to addition of MIX, dexamethasone, and insulin accelerated adipogenesis as shown by an earlier rise in C/EBP α and PPAR γ and enhanced Oil Red O staining (Li et al., 2008). In another study, intermittent forskolin treatment (1 h per day for 6 days) of human bone marrow-derived MSCs inhibited adipocyte differentiation (Rickard et al., 2006). Taken together, it appears that the effect of cAMP on adipogenesis is likely dependent on the level and duration of cAMP signaling (Yarwood et al., 1995; Rickard et al., 2006; Li et al., 2008). The differing effects of cAMP may also be due to cellular compartmentalization of certain kinases and phosphatases with particular receptors (reviewed in McConnachie et al., 2006; Cheepala et al., 2013).

Cyclic-AMP response element binding protein (CREB) has been reported to be necessary and sufficient for adipogenesis in vitro. CREB is constitutively expressed in 3T3-L1 fibroblasts prior to induction of adipogenesis (Reusch et al., 2000). CREB binds cAMP response elements (CREs) and increases transcription of genes important for adipocyte function, including phosphoenolpyruvate, FABP/aP2 and FAS (Reusch et al., 2000). CREB has also been shown to induce C/EBP β and cyclin D1, which promotes mitotic clonal expansion, and reduce expression of Wnt10b, an inhibitor of adipogenesis (Zhang et al., 2004; Fox et al., 2008). Ectopic expression of CREB in 3T3-L1 fibroblasts is sufficient to induce adipocyte differentiation, while expression of a dominant negative CREB prevents adipogenesis (Reusch et al., 2000). Knockdown of CREB with siRNA prevents adipocyte differentiation even in cells ectopically expressing C/EBP β , C/EBP α , or PPAR γ (Fox et al., 2006). However, the CREB knock out (KO) mouse shows no obvious adipose phenotype (Rudolph et al., 1998).

On the other hand, the alpha subunit of G protein, G α s, which activates adenylate cyclase and increases intracellular cAMP levels, has been shown to play an inhibitory role in adipogenesis. The expression of G α s declines with adipogenesis and constitutive expression of G α s inhibits adipogenesis in 3T3-L1 and 3T3-F442A cells via a reduction in PPAR γ (Wang et al., 1992, 1996; Denis-Henriot et al., 1998; Liu et al., 1998; Zhang et al., 2009). Conversely, suppression of G α s expression by antisense oligonucleotides accelerates adipogenesis in the presence or absence of classical inducers (Wang et al., 1992). However, a recent study showed that mice with adipose-specific G α s deficiency have a significant loss of in vivo adipogenesis, as assessed by an almost complete absence of mesenteric and epididymal fat in the knockout mice and very small inguinal fat pads (Chen et al., 2010). Moreover, embryonic fibroblasts from these null mice differentiate poorly to adipocytes even in the presence of MIX, DEX, insulin, and rosiglitazone (Chen et al., 2010). Cholera toxin, which ADP-ribosylates and activates G α s, blocks adipocyte differentiation of 3T3-L1 cells, while pertussis toxin, which also increases intracellular levels of cAMP, does not inhibit adipocyte differentiation (Wang et al., 1992). It has, therefore been suggested that the G α s subunit may play a role in regulating adipogenesis that is independent of activation of adenylate cyclase and elevating cAMP (Wang et al., 1996; Wang and Malbon, 1999).

Calcium Signaling and Adipogenesis

The adenosine receptors are also known to couple to G α q and increase cytoplasmic levels of Ca²⁺. Several studies in 3T3-L1 preadipocytes have demonstrated that high intracellular levels of calcium early in adipogenesis potently inhibit adipogenesis, while increased intracellular calcium in the late stage of adipogenesis promotes differentiation (Miller et al., 1996; Ntambi and Takova, 1996; Shi et al., 2000; Neal and Clipstone, 2002). In one study, the calcium dependent serine/threonine phosphatase, calcineurin was found to be necessary for calcium inhibition of adipogenesis (Neal and Clipstone, 2002). Calcineurin overexpression decreased protein levels of PPAR γ and C/EBP α during adipocyte induction as compared to control, but did not affect protein levels of C/EBP β and C/EBP δ (Neal and Clipstone, 2002). Calcineurin inhibition of PPAR γ and C/EBP α may be mediated by Nuclear factor of activated T-cells (NFAT) (Neal and Clipstone, 2002). NFAT is a transcription factor that upon dephosphorylation by calcineurin translocates to the nucleus. NFAT has been shown to bind to the aP2 promoter during fat cell differentiation and cyclosporin A, an inhibitor of calcineurin, inhibits fat cell differentiation (Ho et al., 1998). Prostaglandin F₂ α binding to its receptor activates G α q protein and inhibits adipogenesis (Liu and Clipstone, 2007). However, PLCdelta1 KO mice have reduced body weight and reduced epididymal fat mass on regular diet or following high fat diet as compared to controls. Adipocytes were smaller and preadipocytes isolated from subcutaneous tissue from these KO mice had a reduced potential to differentiate into adipocytes (Hirata et al., 2011).

Calcium oscillations are believed to confer sensitivity and specificity to pleiotropic calcium signaling. These fluctuations reduce the threshold for calcium activation of transcription factors, while oscillation frequency may activate certain transcription factors like Nuclear factor of activated T-cells (NFAT) (Dolmetsch et al., 1998). Spontaneous intracellular Ca²⁺ oscillations are present in human MSCs due to autocrine/paracrine ATP signaling to P2Y1 receptors (Kawano et al., 2002, 2003, 2006). As adipocytes differentiate, Ca²⁺ oscillations do not occur and NFAT does not translocate to the nucleus (Kawano et al., 2006). During differentiation and proliferation, these Ca²⁺ oscillations may play a large role in regulating the Ca²⁺ signal (Clapham, 1995; Thomas et al., 1996; Berridge et al., 2000, 2003; Schuster et al., 2002).

Conclusion

Signaling molecules, including adenosine, that bind to G-protein coupled receptors represent a novel approach to the obesity epidemic. Pharmacologic agents acting on these receptors may impact adipocyte differentiation. Understanding cell fate decisions could provide new insights into how adipogenesis affects metabolic homeostasis and could represent a viable target for treating or preventing obesity and its associated consequences. Although more mechanistic information is needed, the findings presented in this review highlight the importance of these receptors and ligands in the control of adipogenesis and present a new path to pursue. It appears that receptors coupled to G α i proteins promote differentiation, while receptors coupled to G α s inhibit adipocyte differentiation (Table 1). It is not clear, however, whether these actions are primarily due to activation of cAMP and PKA, or due to other mechanisms, particularly given the conflicting evidence on the role of cAMP in adipogenesis. In addition it appears that receptors coupled to G α q proteins promote

adipogenesis. The ability of a receptor to promote or inhibit adipocyte differentiation may also depend on the receptor density and on when the receptor is expressed during differentiation.

One promising ligand, adenosine, and stable analogs, agonists and antagonists of its associated receptors, particularly the A2aAR and A2bAR, appear to play an important role in stem cell differentiation to various lineages. These receptors would be a good target for drug therapy that might either promote or inhibit differentiation. More details are needed prior to the implementation of a therapeutic strategy to target obesity, however, this area of research could potentially yield significant public health benefits.

Acknowledgements

This work was supported by the National Heart, Lung and Blood Institute Grant (HL93149 to K.R.), and by the Boston Nutrition Obesity Research Center (DK046200 to K.R.), an established Investigator with the American Heart Association. A.S.E. was supported by the Cardiovascular Training Grant from the National Institutes of Health (HL007969) and by the National Institutes of Diabetes and Digestive and Kidney Diseases NRSA F30 award (DK098834). We have no conflicts of interest to disclose.

Contract grant sponsor: National Heart, Lung and Blood Institute;

Contract grant number: HL93149.

Contract grant sponsor: Boston Nutrition Obesity Research Center;

Contract grant number: DK046200.

Contract grant sponsor: National Institutes of Health;

Contract grant number: HL007969.

Contract grant sponsor: National Institute of Diabetes and Digestive and Kidney Diseases;

Contract grant number: DK098834.

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Fig. 1.

Adenosine production and signaling. Adenosine and ATP are released from cells during times of stress, inflammation, and cell damage. ATP can be converted to adenosine by CD39 and CD73 ectonucleotidases. Adenosine can also be released from cells by transporters, ENT1,2. Adenosine binds to receptors on the cell membrane that inhibit (A₁AR and A₃AR) or stimulate (A_{2a}AR and A_{2b}AR) adenylyl cyclase.

TABLE 1

The role of G-protein coupled receptors in adipogenesis

Receptor	Cell type	Effect on adipogenesis	Mechanism	Refs.
Couples to Gas				
A2aAR	MSCs	Promotes	Increase PPAR γ and C/EBP α	Gharibi et al. (2011)
A2bAR	752 cells	Inhibits	Unknown	Gharibi et al. (2012)
β 2-AR/ β 3-AR	MSCs	Inhibits	Via activation of PKA	Li et al. (2010)
TSH receptor	3T3-L1	Inhibits	Decrease PPAR γ due to inhibition of FOXO1	Zhang et al. (2009)
TSH receptor	ESCs	Promotes	Unknown	Lu and Lin (2008)
Couples to G γ q				
P2Y	3T3-L1	Promotes	Alters actin cytoskeleton and induces cell migration	Omatsu-Kanbe et al. (2006)
P2Y1/P2Y4	MSCs	Promotes	Unknown	Ciciarello et al. (2013)
GPR120	3T3-L1, primary preadipocytes	Promotes	Unknown	Gotoh et al. (2007), Ichimura et al. (2012)
Prostaglandin F2 α receptor	3T3-L1	Inhibits	Calcineurin dependent inhibition of PPAR γ and C/EBP α	Liu and Clipstone (2007)
GPR103	3T3-L1	Promotes	Increase fat storage and decrease lipolysis	Mulumba et al. (2010)
Couples to G α i				
A1AR	MSCs, 752 cells, ob17 cells	Promotes	Enhances lipogenesis	Gharibi et al. (2011), Gharibi et al. (2012), Tatsis-Kotsidis and Erlanger (1999a,b)
GPCR43	3T3-L1	Promotes	Unknown	Hong et al. (2005)