JNK- and IkB-dependent pathways regulate MCP-1 but not adiponectin release from artificially hypertrophied 3T3-L1 adipocytes preloaded with palmitate in vitro

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ADIPOCYTES STOCKPILE TRIGLYCERIDES, and increasing adipocyte size or hypertrophy accompanies the increased intracellular triglyceride content (43). In addition to triglyceride stockpiling, adipocytes function in endocrine signaling by secreting adipokines such as adiponectin, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and monocyte chemoattractant protein-1 (MCP-1) (31). Adipokine MCP-1 is a potent chemotactic factor for monocytes and is predominantly produced by macrophages and vascular endothelial cells (34). MCP-1 expression in adipose tissue leads to macrophage infiltration into this tissue and insulin resistance under obese conditions (5, 17, 18). In vivo in obese mice, MCP-1 is abundantly expressed in adipose tissue and elevated in plasma (35). It has been proposed that MCP-1 expression increases due to oxidative stress in adipose tissue under obese conditions (12); the mechanism for this upregulation has not been confirmed.

Unlike MCP-1, adiponectin expression is considered specific to adipose tissue (23). Adiponectin exists in high concentration in normal human serum (27), exerts antiatherogenic effects (28, 51), and enhances the insulin sensitivity of peripheral target tissues (50). Adiponectin expression increases in response to thiazolidinediones and other peroxisome proliferator-activated receptor-γ (PPARγ) agonists. These drugs, which increase adiponectin expression, increase the population of small adipocytes in vivo and decrease that of large adipocytes (52). Therefore, the increased population of small adipocytes might contribute to the detection of increased adiponectin expression; it was reported (8) that adiponectin mRNA was downregulated in hypertrophic adipocytes from mouse models for obesity and diabetes. In addition, a negative correlation exists between body weight and adiponectin expression in human adults (40). However, paradoxically, a positive correlation exists between human neonatal body weight and adiponectin expression (39). The same positive correlation between body weight and plasma adiponectin concentration was also identified in newborn mice (26). Furthermore, in vitro studies in 3T3-L1 adipocytes (25, 43) have demonstrated that thiazolidinedione stimulation enhanced adiponectin expression with increased cell size and triglyceride content. This finding is apparently inconsistent with the reduction of adiponectin expression observed in hypertrophic adipocytes in vivo.

These data leave unclear how MCP-1 and adiponectin are regulated in hypertrophic adipocytes. We sought to investigate how adipocyte hypertrophy, isolated from other factors potentially operational in obesity, regulates MCP-1 and adiponectin

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expression. To do this we used an in vitro model in which mature 3T3-L1 adipocytes were preloaded with palmitate, resulting in artificially hypertrophied mature adipocytes. In obesity, adipocytes are exposed to increased inflammatory cytokine levels and increased reactive oxygen species (12). In our model here we examined the effect of inflammatory cytokines, oxidative stress, and related signaling pathways on MCP-1 and adiponectin levels as a gauge of what regulation occurs under obese conditions. We found that these adipocytokines divergently respond to the TNF-α, IL-1β, JNK, IκB-α, and PPARγ pathways.

MATERIALS AND METHODS

Reagents. Palmitate, oleate, and murine IL-1β were purchased from Wako (Osaka, Japan). Murine TNF-α was obtained from Diaclone Research (Besançon Cedex, France), N-acetyl-cysteine (NAC) and hydrogen peroxide (H₂O₂) from Sigma (St. Louis, MO), bisphenol A diglycidyl ether (BADGE) from Cayman Chemical (Ann Arbor, MI), and SP600125 from AG Scientific (San Diego, CA). BAY 11-7085, JNK-interacting protein-1 (JIP-1) peptide (JNK inhibitor I), BMS-345541, and T0070907 were from Calbiochem (La Jolla, CA). Antibodies against adiponectin were obtained from Chemicon International (Temecula, CA), and antibodies against MCP-1, TNF-α, and IL-1β were from R & D Systems (Minneapolis, MN). We used anti-β-actin antibody from Sigma. Pioglitazone was a generous gift from Takeda Chemical Industries (Osaka, Japan).

Preparation and treatment of 3T3-L1 adipocytes. 3T3-L1 cells were obtained from the cell bank of the Japanese Collection of Research Bioresources (Tokyo, Japan). Cells were seeded and fed every 2 days in DMEM containing 25 mmol/l glucose supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 100 mmol/l MEM sodium pyruvate, and 10% FCS. Cells were grown under 5% CO₂ at 37°C (36, 49). At confluence, differentiation was induced by addition of medium containing 500 μmol/l isobutylmethylxanthine (Sigma), 250 mmol/l dexamethasone (Sigma), and 1.7 μmol/l insulin. After 48 h, this mixture was replaced with fresh medium. The medium was

Fig. 1. Intracellular triglyceride content in the palmitate-preloaded 3T3-L1 adipocytes. Mature 3T3-L1 adipocytes were preloaded with 0.3 mmol/l palmitate (black bars) or ethanol vehicle alone (open bars) for 48 h. At 24 h after the addition of palmitate, 3T3-L1 adipocytes were further treated with either 100 ng/ml TNF-α, 10 ng/ml IL-1β, or sterile distilled water vehicle alone for an additional 24 h. Triglyceride content in cells was measured, and then the concentration was adjusted to the cell number. Results are means ± SE (n = 6). **P < 0.01 compared with corresponding control cells.

Fig. 2. Intracellular and secreted monocyte chemoattractant protein-1 (MCP-1) and adiponectin in 48-h palmitate-preloaded adipocytes. Mature 3T3-L1 adipocytes were preloaded with 0.3 mmol/l palmitate (black bars) or ethanol vehicle alone (open bars) for 48 h, and intracellular MCP-1 (A) and adiponectin (C) protein and their release (B and D, respectively) were analyzed by quantitative immunoblots. β-Actin served as an internal control. A–D, top: representative pictures of immunoblotting that was quantified. Results are means ± SE (n = 4). **P < 0.01 compared with vehicle.
then changed every 2 days until the cells were used for experiments. On day 14 after the induction of adipocyte differentiation, 0.3 mmol/l palmitate was added to the culture medium (the maximum concentration usable without inducing cytotoxicity for the purpose of preparing the artificially hypertrophied mature adipocytes in vitro) (2, 37). At 8 h after the addition of palmitate, MCP-1 release was quantified by immunoblotting. Then, at 24 h after the addition, cells were further treated with either 100 ng/ml TNF-α (11, 38), 10 ng/ml IL-1β (6, 22), or vehicle alone and then cultured for another 24 h to investigate the effect of these exogenous cytokines on MCP-1 and adiponectin release. Triglyceride contents were extracted and measured for adipocytes with or without palmitate loading for 8 h, and also for TNF-α- or IL-1β-treated adipocytes, with or without palmitate loading for 48 h. In brief, cultured adipocytes were washed with PBS three times. Intracellular triglycerides were extracted with isopropanol and measured using Triglyceride E-test (Wako) according to the manufacturer’s protocol. The resultant concentrations were adjusted to the cell numbers. The amount of intracellular or secreted MCP-1, adiponectin, TNF-α, and IL-1β were quantified by immunoblotting. Lactate dehydrogenase (LDH) levels in culture medium were determined by a commercially available ELISA kit (Roche, Mannheim, Germany) according to the manufacturer’s protocol. Like the preloading with palmitate, on day 14 after the induction of adipocyte differentiation, 0.3 mmol/l oleate was added to the culture medium. Then, after 48 h, intracellular triglyceride contents were measured as described above, and MCP-1 and adiponectin release were analyzed by immunoblotting.

Fig. 3. Intracellular triglyceride content and secreted MCP-1 in 8-h palmitate-preloaded adipocytes. Mature 3T3-L1 adipocytes were preloaded with 0.3 mmol/l palmitate (black bars) or ethanol vehicle alone (open bars) for 8 h, and then intracellular triglyceride content was measured (A) and MCP-1 (B) release was analyzed by quantitative immunoblots. β-Actin served as an internal control. B, top: representative picture of immunoblotting that was quantified. Results are means ± SE (n = 4). NS, no significant difference compared with vehicle.

Fig. 4. Intracellular triglyceride content and secreted MCP-1 and adiponectin in 48-h oleate-preloaded adipocytes. Mature 3T3-L1 adipocytes were preloaded with 0.3 mmol/l oleate (black bars) or ethanol vehicle alone (open bars) for 48 h, and then intracellular triglyceride content was measured (A) and both MCP-1 (B) and adiponectin (C) release were quantified by immunoblot analysis. β-Actin served as an internal control. B and C, top: representative pictures of immunoblotting that was quantified. Results are means ± SE (n = 4).
Effects of palmitate preloading and exogenous TNF-α or IL-1β and of oleate preloading on triglyceride contents of 3T3-L1 adipocytes. Although TNF-α was expected to decrease triglyceride levels due to the induction of lipolysis, provision of exogenous TNF-α (100 ng/ml) or IL-1β (10 ng/ml) alone did not affect intracellular triglyceride contents in fully differentiated 3T3-L1 adipocytes. Preloading cells with 0.3 mmol/l saturated fatty acid palmitate for 48 h resulted in a significant, 1.5-fold increase in their triglyceride content (P < 0.01). However, the addition of exogenous TNF-α or IL-1β to the palmitate-preloaded cells did not induce any change in triglyceride content (Fig. 1). Measurement of LDH levels in the culture medium showed no significant changes in LDH among cells incubated with 100 ng/ml TNF-α, 10 ng/ml IL-1β, or vehicle alone under 0.3 mmol/l palmitate (data not shown), indicating that the concentrations used were not cytotoxic.

On the other hand, no significant increase in intracellular triglyceride content was observed by preloading with 0.3 mmol/l palmitate for 8 h and with 0.3 mmol/l unsaturated fatty acid oleate for 48 h, as shown in Figs. 3A and 4A, respectively.

Increased intracellular and secreted MCP-1 and adiponectin in hypertrophied vs. control adipocytes. Immunoblotting results showed that both intracellular and secreted MCP-1 levels significantly increased in the 48-h palmitate-preloaded adipocytes relative to control cells; intracellular MCP-1 increased 1.8-fold, and secreted MCP-1 increased 2.0-fold (P < 0.01; Fig. 2, A and B). Relative to control cells, adipocytes preloaded for 48 h also showed significantly increased intracellular and secreted adiponectin levels (2.1- and 2.0-fold, respectively (P < 0.01; Fig. 2, C and D)). Immunoblotting also revealed that the three multimer formations of adiponectin (45), high molecular weight, middle molecular weight, and low molecular weight, were all secreted in similarly increased levels from preloaded vs. control cells (data not shown). By contrast, no significant effect was elicited by preloading with palmitate for 8 h on MCP-1 release from adipocytes (Fig. 3B) or with oleate for 48 h on both MCP-1 and adiponectin release.

![Image](http://ajpendo.physiology.org/)

**Fig. 5. Intracellular and secreted endogenous TNF-α and IL-1β in palmitate-preloaded adipocytes. Mature 3T3-L1 adipocytes were preloaded with 0.3 mmol/l palmitate (black bars) or ethanol vehicle alone (open bars) for 48 h, and intracellular TNF-α (A) and IL-1β (C) and their release (B and D, respectively) were analyzed by quantitative immunoblots. β-Actin was assessed as an internal control. A–D, top: representative pictures of immunoblotting that was quantified. Results are means ± SE (n = 4). **P < 0.01 compared with vehicle.**
Influence of palmitate preloading on endogenous TNF-α and IL-1β and its release from 3T3-L1 adipocytes. Immunoblot analysis showed that intracellular TNF-α was significantly increased, 1.5-fold \((P < 0.01)\), in the preloaded adipocytes when compared with the corresponding non-preloaded cells. No significant change was observed in the amount of TNF-α secreted (Fig. 5, A and B). This apparent discrepancy might be due to the disordered cleavage of transmembrane TNF-α from fully differentiated adipocytes, as has been demonstrated previously (1, 48). Both intracellular and secreted IL-1β levels showed no significant difference between the hypertrophied and control adipocytes (Fig. 5, C and D).

Effects of exogenous TNF-α and IL-1β on MCP-1 and adiponectin release from preloaded adipocytes. The increased release of MCP-1 from palmitate-preloaded adipocytes was enhanced 17.8-fold by the addition of exogenous TNF-α relative to cells receiving vehicle alone \((P < 0.01)\), as in the case of the corresponding non-preloaded adipocytes \((P < 0.01\); Fig. 6A). However, the addition of exogenous TNF-α conversely inhibited the increased secretion of adiponectin in preloaded cells \((P < 0.01)\), being similar in the non-preloaded cells \((P < 0.05)\), as shown in Fig. 6B.

The increased release of MCP-1 from preloaded adipocytes was also enhanced after addition of exogenous IL-1β; MCP-1 secreted from preloaded cells receiving IL-1β increased 2.6-fold relative to preloaded cells receiving vehicle alone \((P < 0.01)\). A similar pattern was observed in the non-preloaded adipocytes \((P < 0.01\); Fig. 6C). In contrast, as indicated in Fig. 6D, the increased adiponectin secretion seen in hypertrophied adipocytes was significantly suppressed by addition of IL-1β, revealing adiponectin levels similar to those for non-preloaded cells \((P < 0.01)\).

Exogenous H2O2 increases MCP-1 release but reduces adiponectin release from differentiated adipocytes. We next tested the effects of exogenous H2O2 on MCP-1 and adiponectin release from differentiated, non-preloaded adipocytes. Differentiated 3T3-L1 adipocytes were exposed to various concentrations of H2O2 or vehicle alone for 24 h. Treatment with 30, 100, and 300 \(\mu\)M H2O2 dose-dependently increased MCP-1 release by 1.5, 1.9, and 2.0-fold, respectively, relative to control cells receiving vehicle \((P < 0.01\); Fig. 7A). In contrast, adiponectin release was blunted by exogenous H2O2.
addition of 30, 100, and 300 μmol/l H₂O₂ diminished adiponectin secretion in a dose-dependent manner by 20, 42, and 52%, respectively, relative to cells receiving vehicle alone (P < 0.01; Fig. 7B).

We also examined the intracellular concentration of endogenous hydroperoxides in nonpreloaded relative to preloaded cells as a marker for endogenous oxidative stress. Intracellular hydroperoxides were significantly increased in palmitate-preloaded adipocytes by 2.8-fold relative to nonpreloaded cells (5.18 ± 0.22 and 1.88 ± 0.22 U.CARR/μg protein in pre- and control cells, respectively; P < 0.01). However, the antioxidant agent NAC (10 mmol/l) clearly attenuated the elevated content of hydroperoxides in palmitate-preloaded cells by 72% relative to preloaded cells receiving vehicle alone (1.46 ± 0.17 U.CARR/μg protein; P < 0.01).

Effects on MCP-1 release from preloaded adipocytes by drugs that regulate JNK, IkB, and PPARγ pathways. Addition of NAC (10 mmol/l) to preloaded adipocytes suppressed the increased MCP-1 secretion (P < 0.01), whereas addition of exogenous H₂O₂ increased MCP-1 release (Fig. 7A). NAC alone had no effect on MCP-1 release in the nonpreloaded adipocytes (Fig. 8A). Moreover, both SP600125 (10 μmol/l), an inhibitor of JNK, and BAY 11-7085 (20 μmol/l), an inhibitor of IκB-α phosphorylation, suppressed MCP-1 secretion from the palmitate-preloaded cells (P < 0.01). Neither SP600125 nor BAY 11-7085 showed any significant effect on MCP-1 secretion from nonpreloaded cells (Fig. 8B). To confirm these observations, we assayed MCP-1 release from preloaded cells using an alternative JNK inhibitor JIP-1 (5 μmol/l) and an additional IκB-α phosphorylation inhibitor, BMS-345541 (25 μmol/l). Treatment with JIP-1 peptide or BMS-345541 significantly suppressed the increased MCP-1 release from preloaded adipocytes (P < 0.01), but not control adipocytes (Fig. 8C).

We also investigated whether pioglitazone (10 μmol/l) could suppress MCP-1 release but observed no significant effect on MCP-1 release from preloaded adipocytes, although levels did slightly increase (Fig. 9A). As with pioglitazone, treatment with BADGE (100 μmol/l), an antagonist of PPARγ, exhibited no effect on MCP-1 secretion seen in preloaded cells (Fig. 9B). Treatment with T0070907 (1 μmol/l), another antagonist of PPARγ, showed no effect on MCP-1 release from either preloaded or control cells (Fig. 9C).

Adiponectin release is further increased by PPARγ, but not by oxidative stress, in preloaded adipocytes. Adiponectin secretion from preloaded adipocytes was not affected by treatment with NAC (10 mmol/l; Fig. 10A). Similarly, adiponectin secretion from preloaded cells was not affected by treatment with JNK inhibitor SP600125 (10 μmol/l) or IκB inhibitor BAY 11-7085 (20 μmol/l), nor did either drug affect adiponectin release in the nonpreloaded adipocytes, as shown in Fig. 10B. Treatment with the alternative JNK inhibitor JIP-1 peptide (5 μmol/l) or another IκB-phosphorylation inhibitor, BMS-345541 (25 μmol/l), confirmingly did not affect adiponectin secretion from hypertrophied adipocytes nor from nonpreloaded adipocytes (Fig. 10C).

Treatment with pioglitazone (10 μmol/l) further enhanced adiponectin release from preloaded cells 1.3-fold relative to cells receiving vehicle (P < 0.01). In nonpreloaded adipocytes, pioglitazone treatment also induced a 1.9-fold increase in adiponectin secretion relative to vehicle treatment (P < 0.01; Fig. 11A). However, although the PPARγ antagonist BADGE alone had no effect on adiponectin release from the nonpreloaded cells (Fig. 11B), BADGE (100 μmol/l) treatment did significantly reduce adiponectin secretion from preloaded cells (P < 0.01). Another PPARγ antagonist, T0070907 (1 μmol/l), likewise inhibited adiponectin secretion from preloaded adipocytes (P < 0.01), whereas it demonstrated no effect in non-preloaded cells (Fig. 11C).

DISCUSSION

Evidence has accumulated demonstrating that the increased level of MCP-1 in adipose tissue closely relates to the insulin resistance in obese subjects in vivo (30). However, it is still to be clarified how MCP-1 release increases in hypertrophied adipocytes under conditions without infiltrating macrophages. In this study, we designed a hypertrophied adipocyte model by preloading adipocytes with palmitate to yield fully differentiated 3T3-L1 adipocytes. We chose this model on the basis of comparison with physiologically hypertrophied adipocytes...
1. Increased triglyceride content is capable of increasing the cell's diameter parallel with area, reflecting the hypertrophy of adipocytes (43), and 2) the saturated fatty acid palmitate is a physiological component of triglyceride in adipocytes, and it has been reported recently (41) that MCP-1 expression was significantly increased by addition of the saturated, but not by the unsaturated, fatty acid in 3T3-L1 adipocytes. This interesting finding was verified by our observation that the preloading for 48 h with the saturated fatty acid palmitate significantly augmented MCP-1 release from the hypertrophied adipocytes, although the similar preloading with the unsaturated fatty acid oleate did not. Consequently, we investigated the alteration of MCP-1 release from these palmi-
tate-preloaded mature adipocytes relative to control cells under varying conditions and explored the intracellular signaling pathway involved in this alteration.

In our model, secretion of endogenous TNF-α and IL-1β showed no significant changes between control and hypertro-
phied cells. This finding contradicts the previous hypothesis (24) that release of endogenous adipocytokines such as TNF-α and IL-1β increases in hypertrophied adipocytes. However, we found intracellular and secreted MCP-1 to be significantly upregulated in the hypertrophied adipocytes preloaded with palmitate in this study even under conditions without infiltrated macrophages in vitro. We also noted that exogenously admin-
istered TNF-α enhanced MCP-1 release from the hypertrophied adipocytes considerably more than IL-1β. We interpret these data as a possible mechanism whereby TNF-α, increas-
ing MCP-1 release, could worsen insulin resistance in an obese model. TNF-α is presumably released from a large number of infiltrating macrophages recruited by MCP-1 from hypertro-
phied adipocytes. TNF-α and MCP-1 would thus each lead to an increase in each other in a destructive cycle. This specula-
tion is consistent with the notion that macrophages produce the vast majority of TNF-α, whereas mature adipocytes release the majority of other adipocytokines such as adiponectin (10, 42, 47). It would be the important and interesting issue whether this result has been produced specifically in a model of hyper-
trophied adipocytes prepared by preloading with palmitate. The exploration concerning different effects on MCP-1 expres-
sion and release between the saturated and unsaturated fatty acids and their underlying mechanisms in the hypertrophied

![Fig. 8. MCP-1 release in palmitate-preloaded 3T3-L1 adipocytes is suppressed by N-acetyl-
cysteine (NAC), SP600125, BAY 11-7085, JNK-interacting protein-1 (JIP-1) peptide, and BMS-345541. Mature 3T3-L1 adipocytes were preloaded with 0.3 mmol/l palmitate (black bars) or ethanol vehicle alone (open bars) for 48 h. At 24 h after the addition of palmitate, 3T3-L1 adipocytes were further treated with 10 mmol/l NAC (A), 10 μmol/l SP600125, 20 μmol/l BAY 11-7085 (B), 5 μmol/l JIP-1 peptide, 25 μmol/l BMS-345541 (C), or vehicle (sterile distilled water or dimethyl sulfoxide) alone for an additional 24 h. MCP-1 secretion was assayed by quanti-
tative immunoblots. β-Actin was measured as an internal control. A–C, top: representa-
tive pictures of immunoblotting that was quantified. Results are means ± SE (n = 4). **P < 0.01 compared with the corresponding controls.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00279.2008)
adipocytes could resolve this issue. Since it seems likely that triglyceride-derived fatty acids in adipose tissue largely include saturated fatty acids such as palmitate, the observations made here, being applicable only to saturated fatty acids, would be well related to possible phenomena physiologically occurring in hypertrophied adipocytes in vivo.

We observed that the intracellular concentration of hydroperoxides, an endogenous oxidative stress marker, was significantly increased in palmitate-preloaded cells. Addition of exogenous H$_2$O$_2$ augmented the MCP-1 secretion in the mature 3T3-L1 adipocytes, consistent with a previous publication (12). In addition, we found that the release of MCP-1 was decreased by the addition of NAC, a known antioxidant (19), whereas MCP-1 secretion was not affected by PPAR$_{gamma}$/H$_2$53 agonist (pioglitazone) or PPAR$_{gamma}$/H$_2$53 antagonists (BADGE and T0070907). We speculate that the increase in endogenous oxidative stress via NADPH oxidase activation in adipocytes cultured with elevated levels of fatty acids could therefore be an underlying mechanism for the upregulated MCP-1 release from the hypertrophied cells. In vascular cells, it has been confirmed that fatty acids increase the concentration of diacylglycerol, a physiological activator of protein kinase C (PKC), provoking the production of reactive oxygen species through PKC-dependent activation of NADPH oxidase (16, 32). It is possible that this pathway is involved in upregulating MCP-1 release from hypertrophied adipocytes. Furthermore, since the increased MCP-1 release was found to be suppressed by treatment with the JNK inhibitors SP600125 and JIP-1 peptide, and also by the IxB-@/Nuclear factor-@-B (NF-@-B)-dependent pathways are important for upregulating MCP-1 expression. These pathways are likely responding to endogenous oxidative stress as well. It has been observed recently that two NF-@-B binding sites reside in the MCP-1 gene enhancer (44). Appropriate additional experiments would investigate the JNK, NF-@-B, and also possibly the PKC pathway contributions to MCP-1 regulation in more detail.

The finding that adiponectin as well as MCP-1 increased in expression and secretion in hypertrophied adipocytes appears consistent with the positive correlation between body weight and adiponectin expression in adipose tissue in neonatal (39). Similar to our model void of infiltrating macrophages, neonatal adipose tissue would supposedly not yet be invaded with activated macrophages. Therefore, the involvement of endogenous TNF-@ and IL-1B in the regulation of adiponectin expression and its release in hypertrophied adipocytes in vitro.

**Fig. 9. Pioglitazone, bisphenol A diglycidyl ether (BADGE), and T0070907 do not affect MCP-1 release in palmitate-preloaded adipocytes. Mature 3T3-L1 adipocytes were preloaded with 0.3 mmol/l palmitate (black bars) or ethanol vehicle alone (open bars) for 48 h. At 24 h after the addition of palmitate, 3T3-L1 adipocytes were further treated with 10 @mol/l pioglitazone (A), 100 &mol/l BADGE (B), 1 @mol/l T0070907 (C), or dimethyl sulfoxide vehicle alone for another 24 h. MCP-1 secretion was then measured by immunoblotting, with @-actin as an internal control. A–C, top: representative pictures of immunoblotting that was quantified. Results are means ± SE (n = 4). **P < 0.01 compared with the corresponding controls.**
could be negated since no significant changes were observed in the present study in the released amount of these endogenous adipocytokines between hypertrophied and control cells. However, when exogenous TNF-α and IL-1β were administered, these cytokines suppressed adiponectin release almost equally from hypertrophied adipocytes. This evidence indicates that exogenous IL-1β, as well as TNF-α, could reduce the bioavailability of adiponectin. We speculate that in vivo such exogenous cytokines would derive from infiltrating macrophages to decrease adiponectin release, macrophages already being recruited into adipose tissue through increased MCP-1 release via activation of JNK- and IκB-dependent pathways from hypertrophied adipocytes. A reduction in circulating adiponectin by this speculative mechanism could explain the central role JNK signaling in obesity-induced insulin resistance (14). A strategy suppressing the upregulated MCP-1 release from these adipocytes should accordingly be useful for subsequent restoration of the reduced adiponectin release from adipose tissue in obese subjects.

The PPARγ agonist pioglitazone rather enhanced adiponectin release in preloaded cells. This could be explained by the fact that palmitate can activate PPARγ to upregulate adiponectin expression (20). Antioxidant NAC failed to exhibit any effect on the levels of adiponectin released from hypertrophied cells, whereas exogenous H2O2 decreased adiponectin secretion. Neither the JNK inhibitors JIP-1 peptide and SP600125 nor the IκB inhibitors BAY 11-7085 and BMS-345541 showed effect on adiponectin secretion. This suggests that, unlike MCP-1, adiponectin release does not respond to activation of the JNK- or IκB-dependent pathways due to endogenous oxidative stress. The action of palmitate as a PPARγ agonist could be overwhelming the influence of endogenous oxidative stress in terms of adiponectin release. The difference in intracellular/subcellular compartmentalization (13, 15, 29) between endogenous PPARγ and JNK/NF-κB signaling systems could also contribute to these apparently distinct regulations of MCP-1 and adiponectin release.

Summarily, the expression and release of both MCP-1 and adiponectin are increased in artificially hypertrophied adipocytes in vitro by apparently distinct regulatory mechanisms. MCP-1 appears regulated by a JNK/NF-κB-dependent mechanism, whereas adiponectin is modulated by PPARγ and independent of JNK/NF-κB. Suppressing MCP-1 release from hypertrophied adipocytes by inhibition of JNK/NF-κB activation due to endogenous oxidative stress could prevent macro-

**Fig. 10.** NAC, SP600125, BAY 11-7085, JIP-1 peptide, and BMS-345541 show no effect on adiponectin release in preloaded 3T3-L1 adipocytes. Mature 3T3-L1 adipocytes were preloaded with 0.3 mmol/l palmitate (black bars) or ethanol vehicle alone (open bars) for 48 h. At 24 h after the addition of palmitate, 3T3-L1 adipocytes were treated with 10 μmol/l NAC (A), 10 μmol/l SP600125, 20 μmol/l BAY 11-7085 (B), 5 μmol/l JIP-1 peptide; 25 μmol/l BMS-345541 (C), or vehicle (sterile distilled water or dimethyl sulfoxide) alone for another 24 h. Adiponectin release was then quantified by immunoblotting, with β-actin as an internal control. A–C, top: representative pictures of immunoblotting that was quantified. Results are means ± SE (n = 4). *P < 0.05; **P < 0.01 compared with the corresponding controls.
phage infiltration into adipose tissue in vivo and subsequently restore the reduced adiponectin release in obese subjects.

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MCP-1 RELEASE FROM HYPERTRIFIED ADIPOCYTES


