Adipogenic Cell Line TA1: A Suitable Model to Study the Effect of β-Adrenergic Agonists on Lipid Metabolism (43478)

P. S. DICKERSON WEBER,* R. A. MERKEL,*,† AND W. G. BERGEN*,[1]
Growth Biology Program, Departments of Animal Science and Food Science and Human Nutrition,† Michigan State University, East Lansing, Michigan 48824-1225*

Abstract. **The stable adipogenic cell line TA1 was investigated as a potential *in vitro* system to examine effects of β-adrenergic agonists on lipid metabolism at the cellular level. Initial experiments were conducted to establish whether dexamethasone, indomethacin, or both in combination induce rapid differentiation of TA1 preadipocytes to adipocytes. Based on activity of fatty acid synthase, dexamethasone and indomethacin, individually and in combination, were observed to induce differentiation in TA1 cells at different rates (dexamethasone/indomethacin > indomethacin > dexamethasone). Dexamethasone/indomethacin induced complete differentiation in TA1 cells 4 days after confluence, as indicated by increased activity of fatty acid synthase, glycerol-3-phosphate dehydrogenase, and malic enzyme. Finally, mature TA1 adipocytes were treated with various concentrations of isoproterenol and ractopamine to determine the responsiveness of TA1 adipocytes to a β-adrenergic challenge. Glycerol release was increased and fatty acid synthase activity was decreased in a dose-dependent manner for both isoproterenol and ractopamine. These results indicate that fully differentiated TA1 adipocytes may be useful to study direct cellular effects of lipolytic and lipogenic agents on lipid metabolism.** [P.S.E.B.M. 1992, Vol 201]

The phenethanolamine ractopamine ([RAC] 1-(4 hydroxyphenyl)-2-(1 methyl-3(4 hydroxyphenyl) propylamine) ethanol) (Fig. 1) decreases total fat accretion when included in the diet of finishing pigs (1-4). Whether this effect of RAC is mediated via the endocrine system (indirect) or via binding to receptors on porcine adipocytes (direct effect) is not resolved. In isolated rat adipocytes, RAC enhanced lipid mobilization and depressed lipogenesis (5). Similarly, RAC increased lipolysis and reduced lipogenesis in adipocytes isolated from porcine adipose tissue (6-8). Involvement of β-adrenergic receptors has been suggested in this lipolytic and antilipogenic effect of RAC (5). Previous studies (5, 8) on the effect of β-agonists on lipid metabolism in adipocytes involved short (4- to 6-hr) incubation; long-term direct effects of RAC on lipid metabolism in isolated adipocytes have not been investigated.

Chapman *et al.* (9) described the isolation and characterization of a stable adipogenic cell line, TA1, derived from 5-azacytidine-treated 10T1/2 mouse embryo fibroblasts. When subconfluent, TA1 preadipocytes are indistinguishable from their parent cell type, whereas after reaching a growth-arrested state, TA1 cells exhibit morphology characteristic of mature adipocytes, which is apparent by the appearance of lipid droplets. The synthetic glucocorticoid dexamethasone (DEX) accelerates the conversion of TA1 preadipocytes to adipocytes (9, 10). Similarly, the anti-inflammatory drug indomethacin (INDO) also stimulates differentiation of TA1 cells (11). Within 3 days of INDO treatment, virtually all cells contain lipid droplets (11), whereas DEX-treated cells have only begun to differentiate (9). Thus, INDO appears to promote a more rapid and complete conversion of TA1 preadipocytes to adipocytes than DEX.

TA1 adipocytes are capable of lipid mobilization and down-regulation of enzymes involved in triacylglycerol synthesis upon insult by cachectin (tumor necrosis factor) (12). A striking similarity of responses by

[1] To whom requests for reprints should be addressed at Growth Biology Program, Department of Animal Science, Michigan State University, East Lansing, MI 48824-1225.

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TA1 cells to physiological events which occur *in vivo* was noted (12), which suggests that TA1 cells may be a plausible *in vitro* model for studying regulation of lipid metabolism by exogenous agents. Characterization of TA1 cells centers around gene expression during differentiation (reviewed in [13]), with little information on changes of key lipogenic enzyme during and after differentiation. Therefore, the present studies were undertaken to: establish experimental conditions that promote maximal differentiation of TA1 cells based on fatty acid synthase activity; establish when TA1 cells become a fully differentiated adipocyte culture; and determine responsiveness of fully differentiated TA1 adipocytes to β-adrenergic agonists.

Materials and Methods

Cell Culture Conditions. TA1 cells were generously provided by Dr. Gordon M. Ringold (Institute of Biological Science, Syntex Research Laboratories, Palo Alto, CA). Culture dishes (35 mm) were inoculated with 50,000 TA1 cells and grown to confluence (3–4 days) in 2.0 ml of Dulbecco's modified Eagle medium (4,500 units of glucose) (Gibco Laboratories, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum (Gibco) and 10,000 units of gentamicin (Gibco) in a humidified atmosphere of 5% Co_2 and 95% air at 37°C. Unless otherwise indicated, medium was replaced every 3 days during the experiments.

Drug-Induced Adipogenesis. Confluent cells (Day 0) were treated with either DEX (1×10^{-6} *M*) (9, 10), INDO (1.25×10^{-4} *M*) (11) or a combination of DEX and INDO for 48 hr. Cells were harvested for enzyme assays or morphological examination at intervals from Day 0 to Day 12. Untreated cells were utilized as controls.

Enzyme Assays. TA1 cells, at appropriate times, were rinsed with Dulbecco's phosphate-buffered saline (pH 7.4), harvested from culture plates with a rubber policeman, and resuspended in 0.15 *M* KCl containing 5 m*M* 2-mercaptoethanol (14). Cell lysates were prepared with a Polytron homogenizer (Brinkman Corp., Westbury, NY) and cell extract supernatants were harvested by centrifugation at 20,000 *g* for 15 min at 4°C. The resulting supernatants were immediately assayed for glycerol-3-phosphate dehydrogenase (GPD) (EC.1.1.1.8) activity (15, 16) and malic enzyme (ME) (EC 1.1.1.40) activity (17, 18) using a Gilford model no. 252 recording spectrometer (Gilford Laboratories, Inc., Oberlin, OH). Separate aliquots of supernatants were frozen at −20°C for later protein determination (19). A unit of enzyme activity equals 1 nmol of NADH oxidized per min and activity was standardized on a per milligram of protein basis.

For fatty acid synthase ([FAS] EC 2.3.1.85) activity determination, TA1 adipocyte monolayers were rinsed with Dulbecco's phosphate-buffered saline and treated (10 min) with digitonin solution (0.25 *M* sucrose, 17 m*M* MOPS [pH 7.4], 2.5 m*M* EDTA, and digitonin at 0.8 mg/ml) to release cytoplasmic proteins (20). The cytoplasmic protein suspensions were adjusted to 200 m*M* potassium phosphate and clarified by centrifugation at 20,000 *g* for 15 min at 4°C. Supernatants were immediately assayed for FAS activity (21) using a Cary 2200 recording spectrometer (Varian, Sugarland, TX). Protein concentrations were determined using the Folin-Phenol procedure (19) and activity was expressed as units per milligram of protein, as indicated above.

Lipolysis Assay. Lipolysis (glycerol-release) assay medium consisted of basal Eagle medium (Gibco) supplemented with 3% bovine serum albumin and 0.56 m*M* ascorbic acid (22) equilibrated at 37°C with 5% CO_2 and 95% air for 4 hr. TA1 adipocytes were washed twice with basal Eagle medium followed by the addition of the assay medium plus the β-adrenergic agonists. Incubations were performed at 37°C in 5% CO_2 and 95% air for 2 hr (glycerol release was linear during this time frame). After 2 hr, assay medium was removed and stored at −20°C for later analysis of glycerol. TA1 adipocytes were than trypsinized and counted using a hemocytometer. Glycerol was determined with the glycerol enzymatic food analysis kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) according to manufacturer's instructions. Glycerol concentrations are expressed as nanomoles of glycerol released per 10^6 cell/hr.

Compounds. DEX, INDO and isoproterenol (ISO) were obtained from Sigma Chemical Co. (St. Louis, MO). RAC was kindly donated by Lilly Research Laboratories (Eli Lilly and Co., Greenfield, IN).

Statistical Design. For the drug-induced differentiation experiment, means for FAS were analyzed using a two-way analysis of variance and comparisons were conducted using all pairwise comparison (23). Time course experiments were designed as a randomized complete block design and means were analyzed using one-way analysis of variance (23). Responsiveness data

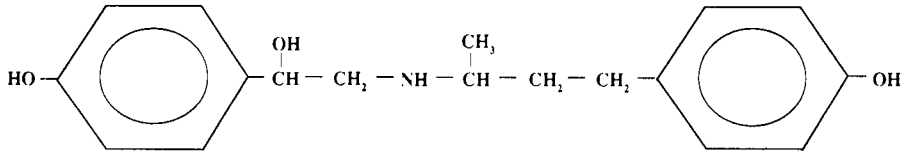

Figure 1. Structure of the phenethanolamine ractopamine.

were analyzed using one-way analysis of variance and comparisons were conducted using Bonferroni's t statistic (23, 24).

Results

Comparison of Drug-Induced Adipogensis. To establish an improved induction system to convert TA1 cells to mature adipocytes DEX and INDO, alone and in combination (DEX/INDO), were studied as potential adipogenic inducers. Cultures of TA1 cells were treated with DEX, INDO, and DEX/INDO for 48 hr after confluence (Day 0) and subsequently harvested for FAS activity determination on Days 6 and 12. A significant treatment × day interaction was observed; therefore, statistical comparisons were confined to within each sampling day (Fig. 2). INDO- and DEX/INDO-treated cells had greater ($P < 0.05$) FAS activity on Day 6 (Fig. 2) compared with control and DEX-treated cells. FAS activity of DEX/INDO-treated cells was also greater ($P < 0.05$) than that for INDO-treated cells. These results indicate that DEX alone is a poor inducer whereas INDO is an effective inducer of TA1 cell differentiation; however, the combination (DEX/INDO) appears to be a more effective accelerator of adipogenesis than either DEX or INDO alone.

FAS activity in INDO- and DEX/INDO-treated cells was also elevated ($P < 0.05$) on Day 12 (Fig. 2) over the untreated or control cells. FAS activity was greater ($P < 0.05$) in DEX/INDO-treated cells than in either the DEX- or INDO-treated cells, but FAS activity of INDO-treated cells, unlike on Day 6, was not different ($P < 0.05$) from the DEX group. These results (Fig. 2) clearly indicate that DEX, INDO, and DEX/INDO treatments induce differentiation of TA1 cells at different rates (DEX < INDO < DEX/INDO). The differentiation effect of these treatments becomes much less evident as time passes, because all cells eventually differentiate.

The morphological characteristics of TA1 cells 6 days after drug treatment are presented in Figure 3. The untreated cells did not contain visible signs of differentiation (i.e., lipid droplets) by Day 6, whereas in the DEX-treated cells and INDO-triggered cells, fat droplets were apparent. Treatment with DEX/INDO produced a uniformly differentiated culture by Day 6 and greater than 90% of the cells contained visible lipid droplets.

Adipogenesis in DEX/INDO-Induced TA1 Cells. The kinetics of increases in GPD, ME, and FAS activities were determined in DEX/INDO-treated TA1 cells (Fig. 4, A and B). GPD, ME, and FAS activity increased between Day 1 and Day 4, and plateaued on Day 4. FAS and GDP activity remained stable between Days 4 and 12, whereas ME showed a steady decline during this time. Cytoplasmic triacylglycerol droplets were apparent in the majority of the cells by Day 3; however,

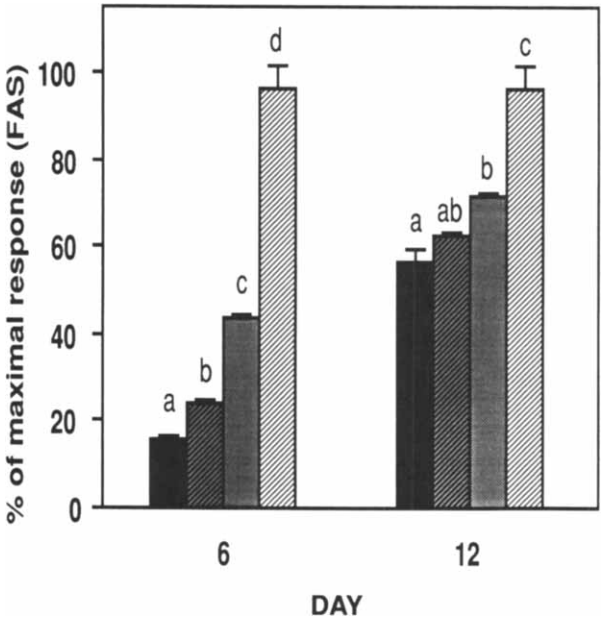

Figure 2. Acceleration of differentiation of TA1 cells. TA1 cells were grown to confluence (Day 0) and treated with 1×10^{-6} *M* dexamethasone (dark diagonal lined bars), 1.25×10^{-4} *M* indomethacin (stippled bars), or both dexamethasone and indomethacin (light diagonal lined bars) or were not treatment (control; solid bars) for 48 hr. Cells were harvested on Days 6 and 12 for fatty acid synthase (activity determination. Values are mean + SE of three independent studies. Different letters indicate differences ($P < 0.05$) within each day. 100% = 19.3 units/mg of protein.

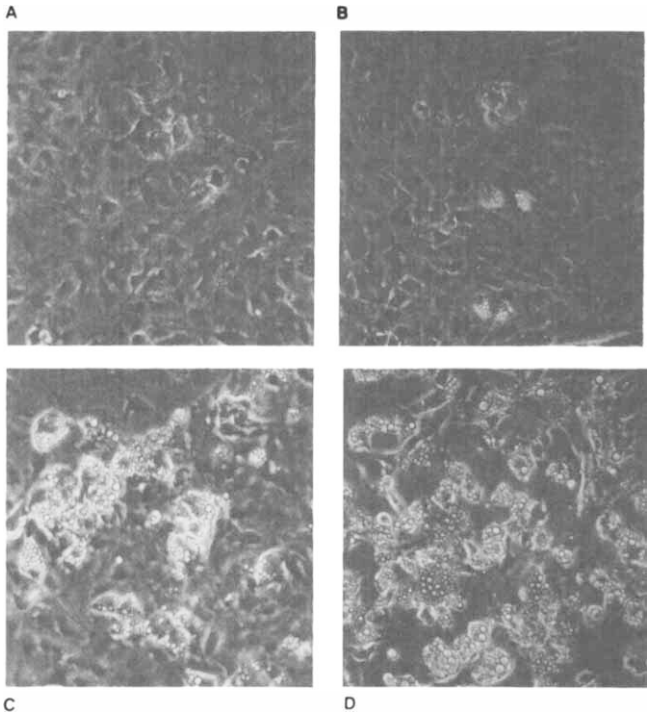

Figure 3. Induction of adipogenesis by dexamethasone and indomethacin in TA1 cells. Confluent TA1 cells were treated with (A) vehicle, (B) 1×10^{-6} *M* dexamethasone, (C) 1.25×10^{-4} *M* indomethacine or (D) dexamethasone and indomethacin combined for 48 hr. The photomicrographs were taken on Day 6.

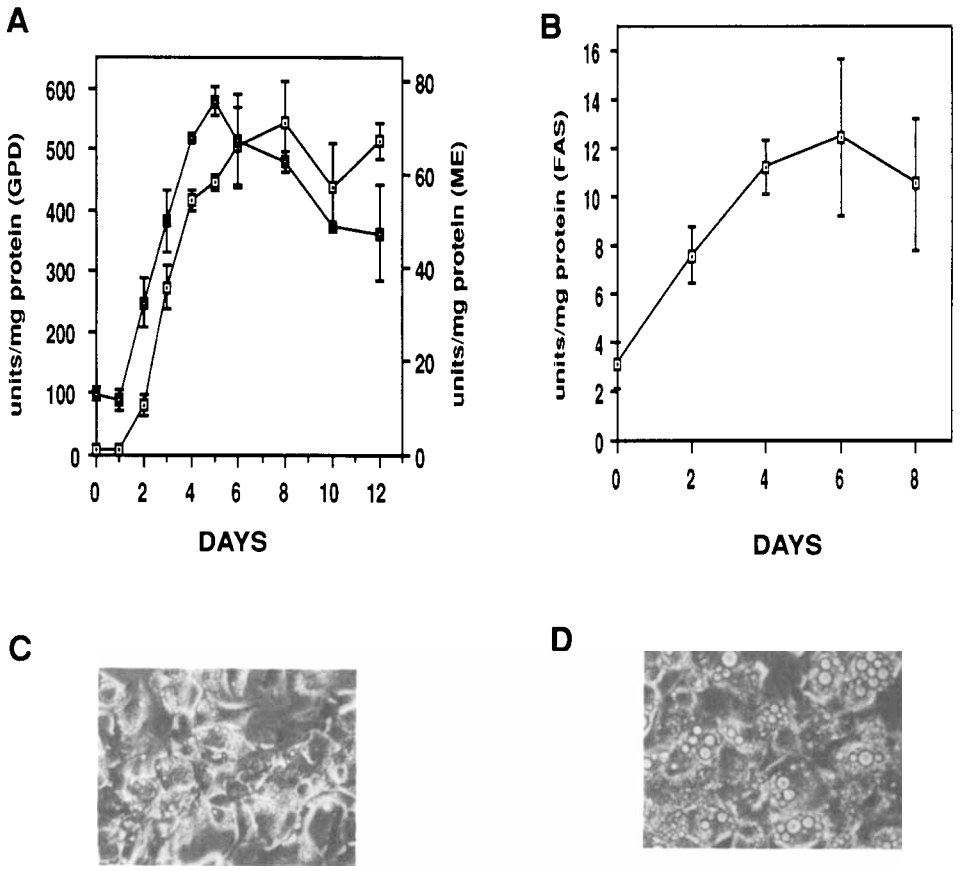

Figure 4. Changes in glycerol-3-phosphate dehydrogenase activity, malic enzyme activity and fatty acid synthase activity in TA1 cells during adipogenesis. Differentiation was induced in TA1 cells by exposure to 1×10^{-6} *M* dexamethasone and 1.25×10^{-4} *M* indomethacin for 48 hr. Cells were harvested at indicated times for enzyme activity determination. (A) Glycerol-3-phosphate dehydrogenase activity (open squares) and malic enzyme activity (closed squares) (values are means obtained from two separate studies). (B) Fatty acid synthase activity (open squares) (values are means compiled from four independent studies). (C) TA1 cells on Day 3; small lipid droplets are apparent in the majority of the cells. (D) TA1 cells on Day 6; large lipid droplets are apparent in the majority of the cells.

the droplets were relatively small and occupied only a small fraction of the cytoplasm (Fig. 4C). By Day 6, greater than 90% of the cells contained lipid droplets that occupied a majority of the cytoplasmic space (Fig. 4D). These data are consistent with a cause and effect relationship between induced activities of key enzymes and triacylglycerol formation, because the induction of the activities of key enzymes involved in fatty acid and triacylglycerol biosynthesis precede the appearance of lipid droplets. Maximal inductions for GPD, ME, and FAS were 84-fold, 6.5-fold, and 4.0-fold, respectively, which indicates that a major reorganization of cellular metabolism occurred during differentiation. These results also illustrate that with DEX/INDO, a stable adipocyte population is established by Day 4. Future experiments addressing regulation of lipid metabolism in differentiated adipocytes may, therefore, be conducted in TA1 cells 4 days after DEX/INDO treatment.

Responsiveness of Adipocytes to Adrenergic Agonists. Studies have shown ISO interacts with β-adrenergic receptor subtypes β_1 and β_2 (25–27), whereas adrenergic receptor preference of RAC has not been reported. Based on the limited data with β-antagonists, RAC appears to react with β-receptors (5). Since β-adrenergic agonists, such as ISO and RAC, stimulate lipolysis and inhibit fatty acid synthesis in isolated adipocytes (5, 8), the lipolytic and antilipogenic effects of ISO and RAC on differentiated TA1 adipocytes were investigated. Results of these experiments are presented in Figures 5 and 6. The minimum concentration of ISO and RAC, following a 24-hr treatment, required to inhibit fatty acid biosynthesis, as indicated by FAS activity, was 10^{-7} *M* ($P < 0.05$). Additional inhibition of FAS activity was not observed with higher concentrations of either β-adrenergic agonist.

Triacylglycerol mobilization, as measured by glycerol release, was enhanced by both ISO and RAC in a dose-dependent manner (Fig. 6). Basal lipolytic activity of TA1 adipocytes (e.g., RAC and ISO at 10^{-9} or 10^{-10} *M*) was about one half of maximal response noted for the two β-adrenergic agonists. ISO stimulated ($P < 0.05$) triacylglycerol mobilization above basal at 10^{-7} *M*, whereas RAC induced ($P < 0.05$) mobilization at 10^{-8} *M*. Maximal stimulation of lipolysis by ISO and RAC

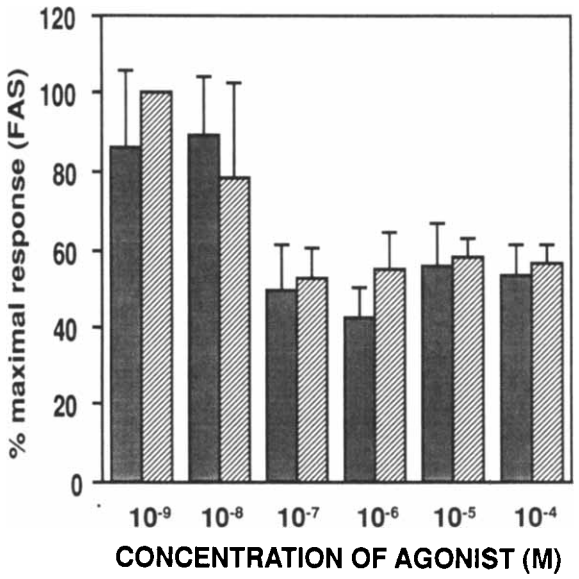

Figure 5. Effect of isoproterenol and ractopamine on fatty acid synthase activity in TA1 adipocytes. Fatty acid synthase activity in isoproterenol- (stippled bars) and ractopamine- (diagonal lined bars) treated TA1 adipocytes. 100% = 8.2 units/mg of protein. Values represent mean + SE from two independent studies.

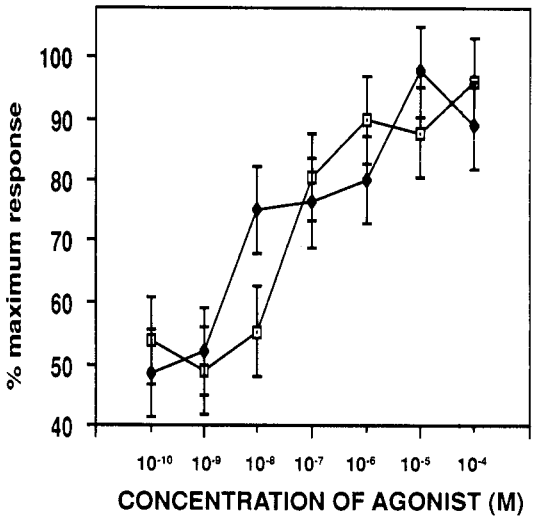

Figure 6. Effect of isoproterenol and ractopamine on lipolytic activity in TA1 adipocytes. Lipolytic activity in isoproterenol- (open squares) and ractopamine- (closed diamonds) treated TA1 adipocytes. Values represent mean ± SE from two independent studies. 100% = 440.0 nmol of glycerol/10^6 cells/hr. Basal rate = 239.6 nmol of glycerol/10^6 cells/hr.

was noted at 10^{-4} *M*, whereas 10^{-3} *M* was toxic to TA1 adipocytes (data not shown).

Discussion

TA1 cells convert to adipocytes *in vitro* (9). Although the conversion to mature adipocytes occurs spontaneously over time in TA1 cells, previous work (9–11) has shown that differentiation can be accelerated by treatment of confluent cultures with either DEX or INDO, with INDO acting as a much more potent initiator. In the present investigation, adipogenesis was accelerated by treatment of confluent TA1 cells with DEX and INDO; however, a more pronounced acceleration was observed when the two drugs were combined, and it appeared to be more than just an additive effect. Similarly, Rubin *et al.* (28) reported that DEX in combination with the cyclic nucleotide phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine proved to be highly effective in promoting differentiation of confluent 3T3-L1 cultures more so than either compound alone.

INDO is recognized as potent inhibitors of prostaglandin synthesis (29–31), and inhibition of prostaglandin synthesis may play a role in triggering the activation of the differentiation program (32–34). In TA1 cells, INDO inhibits prostaglandin production, but at concentrations far lower than those required to stimulate cellular differentiation (11). Therefore, in TA1 cells, INDO inhibition of prostaglandins does not appear to be involved in differentiation of preadipocytes.

Knight *et al.* (11) proposed that an activating substance is responsible for initiating differentiation of preadipocytes. Once preadipocytes reach confluence, this activating substance begins to accumulate and, upon reaching a critical concentration, it promotes cellular differentiation. Inducers of differentiation (i.e., DEX and INDO) potentially perturb this putative mechanism by increasing the synthesis rate of the substance or lowering the threshold for the substance required for differentiation (11). The acceleration of adipogenesis observed in the present investigation can be explained by this hypothetical activating substance, because the rates of induction between each treatment were different but the final levels of differentiation were comparable. Until the putative activating substance has been identified, this entire concept will remain conjectural.

Sequential changes in the activity of three enzymes involved in lipid biosynthesis (GPD, ME, and FAS) were determined to follow the differentiation/maturation of TA1 cells during DEX/INDO-induced differentiation. The present investigation showed that the addition of DEX/INDO resulted in a coordinated induction of FAS, ME, and GPD activity. Enzyme activity began to increase on Day 2 and approached a plateau on Day 4, and differentiation appeared complete by 5 days after confluency (Day 0). Differentiated TA1 cells continued to exhibit a stable adipocyte phenotype for an additional 5 days. Maximal assayable FAS activity in the present work was similar to FAS activity in spontaneously differentiated 3T3-L1 cells (35), or cells differentiated with methyl isobutylxanthine, DEX, or insulin (18) and by methyl isobutylxanthine and DEX (36). Changes recorded by Kuri-Harcuch and Green (37) were similar to those observed in the present study. In previous work, GPD activity increased more than

100-fold during differentiation of the adipogenic cell line TA1 (9), whereas, in the present study, an 84-fold increase in GPD activity was obtained in cells triggered by DEX/INDO. Thus, maximal assayable GPD activities were similar between previous (9) and present work.

A two-phase process in TA1 cell conversion was noted in the present study. An initial exponential phase was characterized by conversion of TA1 cells to adipocytes with rapid changes in key lipogenic and triacylglycerol enzyme activities, while a second phase was characterized by stable enzyme activities that suggest that cultures have become a mature adipocyte population. Regulation of differentiation of adipogenic cells may be studied during phase 1 and long-term regulation of lipid metabolism may be studied in the second phase. Since our overall objective was to utilize the TA1 adipogenic cell line as an *in vitro* model to study the regulation of lipid metabolism in fully operational and mature adipocytes, our studies were conducted on phase 2 adipocytes.

Previous work showed that ISO and RAC enhanced triacylglycerol mobilization and inhibited FAS activity (reviewed in [38]). In the present study, ISO and RAC enhanced triacylglycerol mobilization and inhibited FAS in a dose-dependent manner. These data clearly indicate that fully differentiated TA1 cells can be used as an *in vitro* model to further investigate the cellular effects of β-adrenergic agonists like ISO and RAC on lipid metabolism.

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