Pharmaceutical Research, Vol. 15, No. 6, 1998 Research Paper

Development of Infrared Imaging to Measure Thermogenesis in Cell Culture: Thermogenic Effects of Uncoupling Protein-2, Troglitazone, and β-Adrenoceptor Agonists

Mark A. Paulik,[1,4] Richard G. Buckholz,[2] Mary E. Lancaster,[1] Walter S. Dallas,[3] Emily A. Hull-Ryde,[1] James. E. Weiel,[1] and James M. Lenhard[1,4]

Received December 23, 1997; accepted March 18, 1998

Purpose. Although the effects of thermogenic agents in cell culture can be measured by direct microcalorimetry, only a few samples can be analyzed over several hours. In this report, we describe a robust non-invasive technique to measure real-time thermogenesis of cells cultured in microtiter plates using infrared thermography.
Methods. Yeast were transformed with uncoupling protein-2 (UCP2) or exposed to carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (FCCP) or rotenone. Adipocytes were exposed to rotenone, FCCP, cycloheximide, troglitazone, or CL316243. Thermogenesis was measured using infrared thermography.
Results. Thermogenesis increased after exposing yeast to the mitochondrial uncoupler, FCCP, or transforming the cells with UCP2. Further, thermogenesis in adipocytes was stimulated by CL316243, a β_3-adrenoceptor agonist being developed to treat obesity. The protein synthesis inhibitor, cycloheximide, did not inhibit CL316243-mediated thermogenesis. In contrast, the mitochondrial proton transport inhibitor, rotenone, inhibited thermogenesis in yeast and adipocytes. Similarly, the antidiabetic agent, troglitazone, suppressed thermogenesis in adipocytes. Although increased UCP synthesis resulted in increased thermogenesis in yeast, UCP expression did not correlate with thermogenesis in adipocytes.
Conclusions. The results, taken together with the high resolution (0.002°C) and robustness (384-well format) of the approach, indicate infrared-imaging is a rapid and effective method for measuring thermogenesis *in vitro*.

KEY WORDS: infrared; thermogenesis; adipocyte; uncoupling protein; β-adrenoceptors; troglitazone.

INTRODUCTION

In rodents, brown adipose tissue (BAT) is an important site for adaptive thermogenesis (1). This tissue contains abundant mitochondria which express the anion transporter, uncoupling protein (UCP1, formerly known as UCP) (2). UCP1 uncouples oxidative phosphorylation from respiration in BAT resulting in generation of heat instead of ATP. Although UCP1 is not abundant in *Homo sapiens*, UCP2 (3) is abundantly expressed in humans. UCP2 mRNA is ubiquitously expressed and its expression is altered in obesity (4). Further, the antidiabetic thiazolidinediones (*e.g.*, troglitazone) increase UCP2 expression, possibly by activating the nuclear receptor PPARγ, suggesting UCP2 plays a critical role in regulating energy balance (5).

Catecholamines are postulated to regulate body temperature and composition (6), possibly by regulating UCP expression (7) or activity. In adipocytes, catecholamines activate β-adrenoceptors (β-ARs) resulting in stimulation of the intracellular cAMP pathway (8). Stimulation of the β-AR pathway by norepinephrine injection in animals mimics cold-induced increases in UCP1 mRNA expression in BAT (9). Similarly, norepinephrine (10) and β_3-AR agonists (11) directly stimulate UCP1 expression in cell culture. Thus, it is often assumed that the thermogenic effects of catecholamines and β_3-AR agonists are mediated by increased UCP expression. However, it has yet to be determined if β_3-AR agonists induce thermogenesis in the absence of increased UCP expression.

Various methods are available for detecting βAR- activity or UCP expression, although none of these methods satisfactorily measure heat production in cell culture. Methods such as Northern or Western-blotting have the disadvantage of both being labor intensive and not offering a direct measure of protein activity. Although GDP-binding assays are used as a direct measure of UCP activity (12), this technique requires protein purification, making the procedure time consuming. The advent of fluorescent dyes (*e.g.*, JC-1 or rhodamine derivatives), which accumulate in mitochondria as a function of membrane potential, has overcome some of these difficulties (13). However, these dyes have the disadvantages of high background (*i.e.*, non-selective) staining, cytotoxicity, and becoming metabolized by the cells. More importantly, all of these techniques fail to directly measure real-time fluctuations in thermogenesis.

Although infrared thermography is used to measure heat dispersion from the skin of living animals (14,15), similar techniques have not been developed for detecting temperature changes using isolated cells. Indeed, *in vivo* studies of thermogenesis are limited because of the contributions that multiple tissues make to energy expenditure. We present here for the first time the use of a high resolution infrared imaging system to study reconstitution of heat production in yeast and adipocytes cultured in microtiter plates. This report provides evidence that infrared thermography can be used to measure the thermogenic effects of both anti-obesity (*e.g.*, CL316243) and anti-diabetic (*e.g.*, troglitazone) agents in cell culture. This novel approach provides the biologist with a rapid method for studying how multiple drug candidates affect thermogenesis in isolated cells.

MATERIALS AND METHODS

Adipocytes

Human subcutaneous adipocytes were purchased from Zen-Bio, Inc. (Research Triangle Park, NC). C3H10T1/2 clone 8 fibroblasts were differentiated into adipocytes as previously described (16,17). After 7 days in culture, triglyceride accumu-

[1] Department of Metabolic Diseases, GlaxoWellcome Inc., 5 Moore Drive, Research Triangle Park, North Carolina 27709.
[2] Department of Molecular Endocrinology, GlaxoWellcome Inc., 5 Moore Drive, Research Triangle Park, North Carolina 27709.
[3] Department of Molecular Sciences, GlaxoWellcome Inc., 5 Moore Drive, Research Triangle Park, North Carolina 27709.
[4] To whom correspondence should be addressed. (e-mail: jml29514@glaxowellcome.com; map16209@glaxowellcome.com)

ABBREVIATIONS: UCP, uncoupling protein; FCCP, carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone; AR, adrenoceptor; BAT, brown adipose tissue.

0724-8741/98/0600-0944$15.00/0 © 1998 Plenum Publishing Corporation

lation was determined by adding lipoprotein lipase and GPO-Trinder reagent (assay kit 337-B, Sigma Diagnostics, St. Louis, MO) to the cells (50 μl/cm^2) and incubating the lysates at 37°C for 2 hours. The optical density was measured using a spectrophotometer set at a wavelength of 540 nm. Lipolysis was measured as previously described (16).

Cloning of UCP2 and Yeast Transformation

Human skeletal muscle cDNA (#7175-1) was purchased from Clontech (Palo Alto, CA). UCP2 specific-sequences were PCR amplified from the sample using oligonucleotide primers that matched the 5′ and 3′ ends of a published sequence (GenBank U82819). Vent polymerase was used (New England Biolabs, Beverly, MA) in a standard reaction mixture with 2 mM $MgSO_4$ and 5% DMSO. The cycle parameters were 94°C for 1 min., 55°C for 1 min., and 72°C for 1 min., repeated 29 times. The sample was passed over an S-400 spin column (Pharmacia, Piscataway, NJ) prior to ligation in a vector for transformation of E. coli. The authenticity of the clone was confirmed by DNA sequence analysis. For the experiments in yeast, the UCP2 coding sequence was amplified by PCR using primers with the sequence AAAAAACCCCGGATCGAATTCATGGTTGGGT TCAAGGCCA (sense) and CATTGTTCCTTATTCAGTTAC TCGAGTTAGAAGGGAGCCTCTCGGGA (antisense) followed by a second PCR using primers with the sequence TTAACGTCAAGGAGAAAAAACCCCGGATCG (sense) and GAAAGGAAAAACGTTCATTGTTCCTTATTCAG (antisense). The PCR product was cloned into pYX233 (R&D Systems) by homologous recombination in yeast strain W303 (a/α homozygous for ade2-1 his3-1,15 leu2-3, 112 trp1-1 ura3-1). Yeast transformants were selected on BSM-trp agar (Bio 101, Vista, CA). The correct UCP2 sequence was verified by sequencing plasmids back-extracted from yeast to E. coli.

For analysis of UCP2 expression and thermogenesis, yeast containing the expression plasmids were propagated for 24 hours in BSM-trp broth, washed once and inoculated at A600 = 001 into YP containing 2% DL-lactic acid, pH 4.5, 1% ethanol, 0.1% casamino acids and 40 μ/ml adenine. Cultures were induced 16 hours later by adding galactose to 0.4% (w/v). To assess UCP2 expression, yeast (A600 = 0.1) were lysed in NuPAGE sample buffer (Novex, San Diego, CA) containing 5% β-mercaptoethanol and soluble proteins separated on 10% NuPAGE gels (Novex, San Diego, CA). A synthetic peptide using the UCP2 amino acid sequence LKRALMAAYQS-REAPF was synthesized and used to generate antibodies through a contract with Zeneca Inc., (Wilmington DE).

Western blot analysis was performed following published methods (17).

Infrared Thermography

Heat generation was measured using a thermo-electrically cooled Agema Thermovision 900 Infrared System AB (Marietta, GA) equipped with a SW Scanner and 40° × 25° lens which detects a 2–5.4 micron spectral response. The scanner had an internal calibration system with an accuracy of 0.08°C. The focal distance was 6 cm. Images were captured using a recurs function set at 16. The data was analyzed using OS-9 advanced systems and ERIKA 2.00 software according to the manufacturer's specifications (Agema Infrared Systems AB, Danderyd, Sweden). Thermography of adipocytes was performed by maintaining the ambient temperature of the cultured cells at 37 ± 0.02°C using a Queue Systems Inc. (Parkersburg, W.V.) incubator, model QWJ500SABA. Spectral analysis of yeast was performed at 30 ± 0.02°C using the same incubator system. After treating the cells with the various experimental agents (*e.g.*, rotenone etc.), the temperature was equilibrated for 10 minutes in the incubator before measuring real-time thermogenesis. Various color scales in the visible wavelength were used to depict the temperature fluctuations of the recorded images. Although temperature scales are constant, the color scale images are variable and can be adjusted with level and span controls to display the operators' preference.

RESULTS AND DISCUSSION

Infrared Thermography in Yeast and Adipocytes

As part of the initial effort to validate the use of infrared thermography to monitor mitochondrial heat production, we treated human adipocytes cultured in 96-well microtiter plates and yeast suspended in 384-well microtiter plates with rotenone or carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (FCCP). Rotenone is an inhibitor of mitochondrial electron transport (18) while FCCP is an uncoupler of mitochondrial respiration (19). Subsequently, we measured the heat produced from the cells using an Agema Thermovision 900 Infrared Imaging System (Fig. 1). As shown in the dose response assays in Fig. 2, rotenone treatment inhibited thermogenesis in human adipocytes (Fig. 2A) and yeast (Fig. 2B). In contrast, FCCP stimulated heat production in both cell types relative to untreated cells (Fig. 2). Kinetic analysis revealed that changes in heat production were detectable 10 minutes after treating the cells with either agent (data not shown). Shorter times could

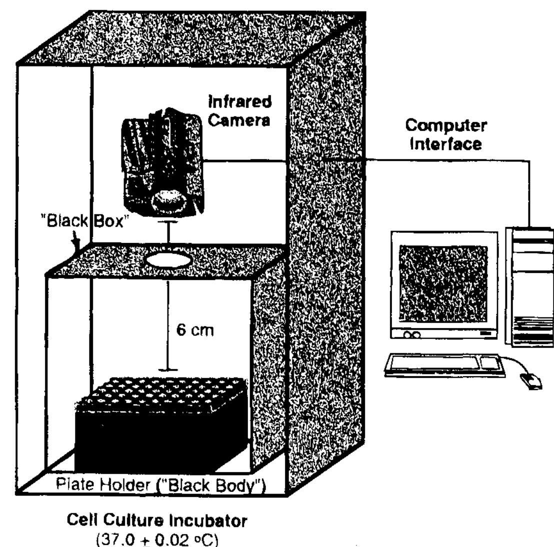

Fig. 1. Apparatus for measuring infrared thermography in cell culture. The "black box" and "black body" minimize thermal noise (*i.e.*, reflection and air currents) from the culture plates and surrounding environment. Use of an incubator also prevents fluctuations in the surrounding temperatures and improves cellular responses and viability. The camera monitors real-time heat production from the cells in culture with images recorded by a central processing unit for further data analysis.

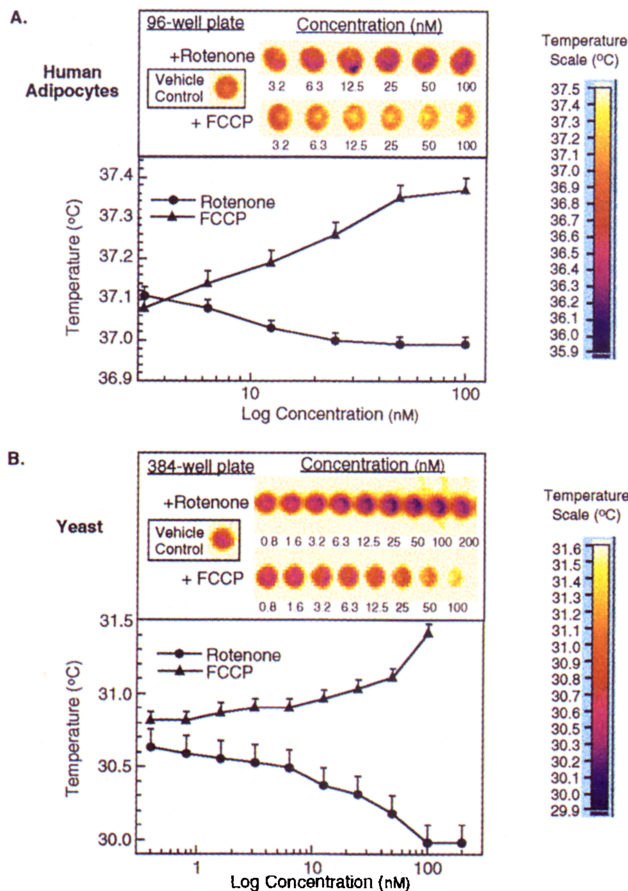

Fig. 2. Infrared image of yeast and human adipocyte cultures treated with rotenone and FCCP. Yeast were suspended to A600=0.1 in 384 well microtiter plates (50 μl/well). Confluent human adipocytes were cultured in 96-well microtiter plates (50 μl/well). Dose responses are shown on the thermogenic effects of rotenone and FCCP on (A) human adipocytes and (B) yeast. The cells were treated for 10 minutes with either agent before the images were analyzed by infrared thermography. Representative data is presented from experiments performed in triplicate.

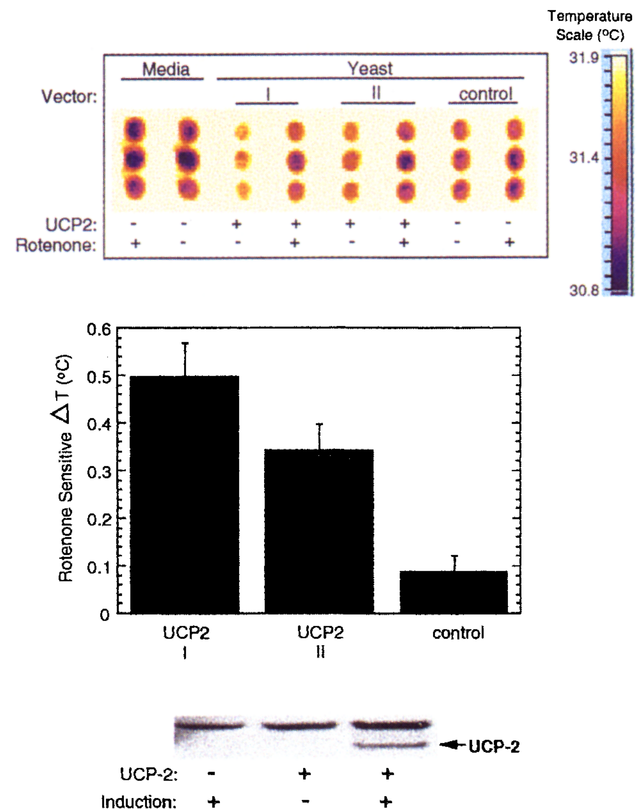

Fig. 3. Thermographic analysis of yeast expressing UCP2. Yeast were transformed with two plasmids expressing UCP2 (isolates I and II) or vector lacking UCP2 (control). The cells were treated for 4 hours with galactose to induce expression and suspended to A600=0.1 in 384-well microtiter plates (50 μl/well). (A) As a control, the cells were treated with (+) or without (−) rotenone for 10 minutes prior to the plates being analyzed by infrared thermography. (B) The rotenone-sensitive thermogenic response (ΔT) was calculated by subtracting the temperature of the cells in the presence of rotenone from the temperature of the cells in the absence of rotenone. (C) For reference, Western analysis of UCP2 was performed on nontransfected cells (UCP2: −) or transfected cells (UCP2: +) which were treated with (induction: +) or without (induction: −) galactose. The upper band is an unidentified yeast protein that is detected by the rabbit anti-serum. Representative data from three experiments is given. Each sample was repeated in triplicate for the given experiment.

not be measured because the system required 10 minutes for temperature equilibration after treating the cells with either agent.

Although UCP1 regulates mitochondrial-mediated thermogenesis in rodents, there is no direct evidence that UCP2 plays a similar role. In order to evaluate UCP2's role in thermogenesis and further validate the use of infrared thermography, the UCP2 gene was cloned from a human cDNA library and expressed in yeast using a galactose-inducible expression system. As shown in Fig. 3A, expression of UCP2 in yeast resulted in increased thermogenesis relative to cells lacking UCP2. As expected, treatment of the cells with rotenone inhibited UCP2-mediated thermogenesis (Fig. 3A and 3B). For reference, we confirmed UCP2 was expressed in these cells by Western blot analysis (Fig. 3C). Peak expression and thermogenesis was observed 3–4 hours after inducing UCP2 synthesis with galactose (data not shown). Taken together, the results validate using infrared thermography as a tool to measure the effects of various molecules (*e.g.*, rotenone, FCCP, and UCP2) on mitochondrial-mediated thermogenesis.

Infrared Analysis of Cells Treated with PPARγ and β-AR Agonists

We were interested in determining if infrared thermography could be used to analyze the pharmacological effects of drugs which alter fuel metabolism. Troglitazone is an antidiabetic agent that increases anabolism (*e.g.*, lipogenesis and mitochondrial mass) and decreases catabolism (*e.g.*, basal lipolysis and aerobic respiration) in C3H10T1/2 cells (16). The effects of troglitazone on these cells is a result of activation of the transcription factor PPARγ which, in turn, induces differentiation of the stem cells into adipocytes (16,17,20). Thus, infrared thermography was used to test the affects of troglitazone and 5 structurally related agonists on heat production in C3H10T1/2 cells (Fig. 4). We also measured the affects of these agents on cellular triglyceride accumulation as a marker for adipogenesis. As shown in Fig. 4A, troglitazone treatment increased

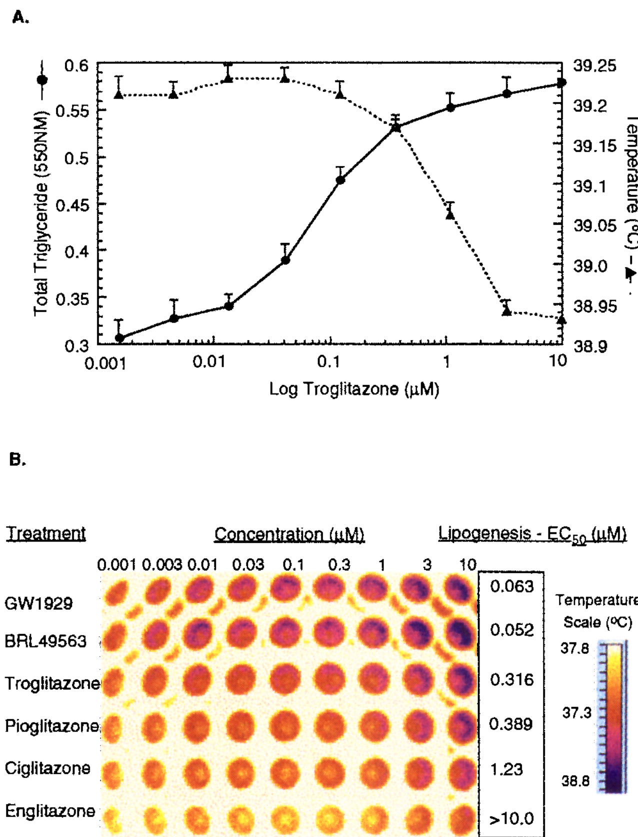

Fig. 4. Infrared image of differentiating adipocytes. Confluent C3H10T1/2 stem cells were cultured in 96-well microtiter plates. (A) Triglyceride accumulation and heat production were analyzed as described in the methods. (B) An infrared thermographic image was recorded one week after treating the cells with increasing concentrations of various PPARγ agonists in the presence of insulin and 9-*cis* retinoic acid. Each data point is the mean ± SD from three replicates.

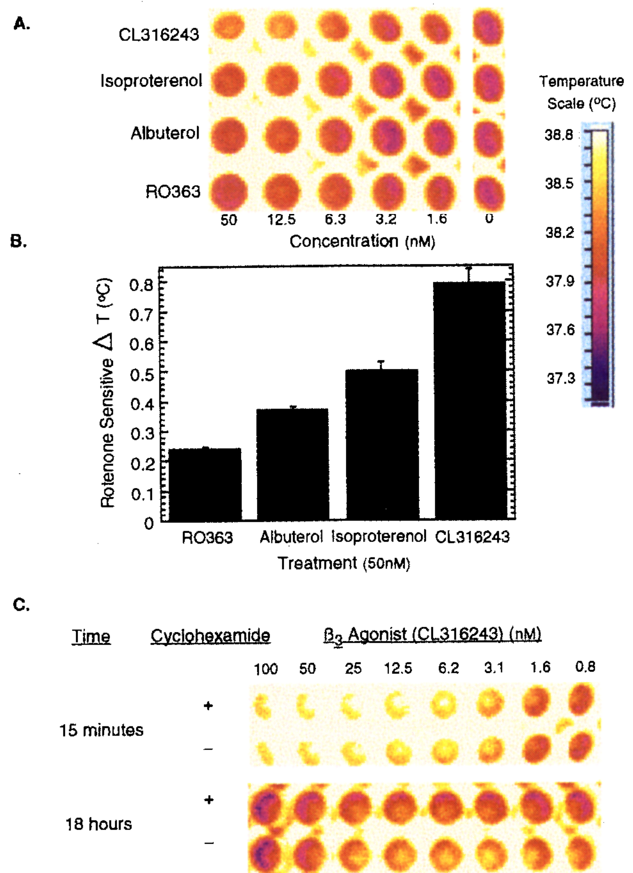

Fig. 5. Effect of β-AR agonists on thermogenesis in adipocytes. C3H10T1/2 cells were maintained in growth medium for 7 days in the presence of 1 μM insulin, 1 μM 9-*cis* retinoic acid, and 1 μM troglitazone to induce adipogenesis. (A) Confluent adipocytes (50 μl/well) were incubated with various concentrations of the given β-AR agonists for 10 minutes and real-time images were recorded by infrared thermography. (B) The rotenone-sensitive thermogenic response (ΔT) was calculated by subtracting the temperature of the cells incubated in the presence of rotenone (100 nM) and β-AR agonists (50 nM) from the temperature of the cells incubated in the presence of only β-AR agonists. (C) The adipocytes were treated with the given concentrations of CL316234 for 15 min and 18 hrs in the presence or absence of 1 μM cycloheximde. At the end of each incubation time, the images were recorded by infrared thermography. Representative data are given from one of three separate experiments.

triglyceride accumulation in these cells, consistent with the observation that this drug promotes adipogenesis (16,20). In contrast, heat production decreased in cells treated with increasing concentrations of troglitazone or the other related PPARγ agonists (Fig. 4B), suggesting thermogenesis is suppressed as these cells differentiate into adipocytes. Further, the rank order potency of the various PPARγ agonists tested in the thermogenesis and lipogenesis assays were BRL49653 ≥ GW1929 > troglitazone ≥ pioglitazone > ciglitazone > englitazone (Fig. 4B). These results indicate infrared thermography can be used to study the pharmacological effects of troglitazone and related PPARγ agonists on heat production and adipogenesis. Moreover, since UCP expression increases as stem cells differentiate into adipocytes (17,21), these observations suggest that increased UCP expression is not sufficient for stimulation of thermogenesis in adipocytes. This finding is in agreement with the suggestion that in addition to increased UCP expression other signals (*e.g.*, β-AR stimulation) are needed to stimulate thermogenesis in adipocytes (16,17).

The β_3-AR agonists are candidate therapeutic agents for the treatment of diabetes and obesity. The mechanism of action for these agents is thought to involve increased metabolic rate (22). Therefore, we tested if infrared imaging could be used to monitor the effects of β-AR agonists on thermogenesis in cultured adipocytes. As shown in Fig. 5A, thermogenesis in C3H10T1/2 adipocytes was stimulated by treatment with the selective β_3-AR agonist, CL316243, and the non-selective β-AR agonist, isoproterenol. The β_1-AR agonist, RO363, and the β_2-AR agonist, albuterol, were less effective than CL316243 or isoproterenol at stimulating thermogenesis in these cells. In contrast to β-AR agonists, the mitochondrial electron transport inhibitor, rotenone, inhibited thermogenesis of cells treated with 50 nM of the various β-AR agonists (Fig. 5B). An inhibitor of protein synthesis, cycloheximide, had no effect on β_3-AR-mediated thermogenesis in these cells (Fig. 5C). Furthermore, thermogenesis was greater after 15 min than after 18 hours treatment with CL316243 (Fig. 5C). Thus, β_3-AR-induced thermogenesis may be an acute response that does not require

increased protein (*e.g.*, UCP) synthesis. However, as suggested by others, these results do not preclude a role for β_3-AR in regulating UCP synthesis (7,8,9).

As a control, the effects of β-AR agonists on lipolysis was also measured. Dose response analysis revealed the various βAR agonists had similar EC_{50}s in both the thermogenesis and lipolysis assays (Table 1). A correlation coefficient of 0.99 was observed when the potencies of the various β-AR agonists were compared in both assays, suggesting the same β-AR-signaling pathways mediate lipolysis and thermogenesis in adipocytes. Taken together, these observations indicate infrared thermography is useful for characterizing how anabolic (*e.g.*, troglitazone) or catabolic (*e.g.*, CL316243) drugs affect heat production in cell culture.

Optimization

Various infrared detection system parameters were optimized using both yeast and cultured adipocytes. Cell titer experiments using yeast revealed a lower detection limit at a density of A600 = 0.01 and a maximal thermogenic response at a density of A600 = 0.1–0.2. Whereas thermal activity of C3H10T1/2 cell suspensions was detected as low as 8×10^3 cells/well, the greatest signal was obtained at 1×10^5 cells/well. As expected, the maximal thermogenic response of adherent adipocytes was observed using confluent cells. A comparison of microtiter plates revealed 384-well formats were best for measuring thermogenesis of yeast suspensions while 96-well formats were most suitable for culturing adherent adipocytes. The outer wells of the culture plates were omitted from the detection system because increased thermal conductance occurred at the edge of the plates. Larger diameter wells (*i.e.*, >1 cm) were less satisfactory because a meniscus effect was observed that resulted in uneven thermal conductance. Further, the amount of media per well was critical, since too much media decreased the signal and too little media created a meniscus resulting in increased background noise. The best results were obtained using 50 μl/well in both 96-well plates containing adherent adipocytes and 384-well plates containing yeast suspensions. Enclosure of the detection system was essential for minimizing changes in temperature and reflectivity (*i.e.*, thermal noise) which result from air currents and light, respectively. Finally, increasing the temperature equilibration time (*i.e.*, > 10 min) improved the signal to noise ratio.

CONCLUSIONS

The pleiotropic effects of multiple tissues on heat dispersion *in vivo* necessitates the measurement of thermoregulatory properties of isolated cells. To address this problem, in part, microcalorimetry has been developed as a technique to measure the heat produced by isolated cells (23). This technique has the advantage of measuring heat output quantitatively (*i.e.*, kJ/mol). However, this technique is limited because it can not be used to measure temperature gradations over fixed surface areas, such as those found in cell culture plates. Thus, we developed several applications for using infrared thermography to measure thermogenesis in cultured cells. Taken together, the data show infrared thermography can be used to measure changes in mitochondrial metabolism in yeast and the pharmacological effects of PPARγ and β-AR agonists in adipocytes. We have observed that infrared thermography has several advantages when compared to other metabolic assays for PPARγ agonists (*e.g.*, lipogenesis assays) or β-AR agonists (*e.g.*, cAMP measurements). For example, this technique does not require any radioactive precursors, thereby eliminating unnecessary exposure to ionizing radiation. Further, it does not require consumable supplies (*e.g.*, substrates or buffers) outside of those needed to maintain cells in culture. Indeed, the non-invasive nature of infrared thermography allows for multiple serial measurements for as long as cells are maintained in culture. Although we did not calculate the rate of change in thermogenesis, it is possible to perform kinetic assays for measuring receptor down regulation using this technique. Finally, it is concluded that the sensitivity (0.002°C) and robustness (*i.e.*, 384-well format) of this detection system will make it a useful tool for analyzing the effects of a variety of agents on heat production in various cell types. Thus, this approach may be used to identify novel agents for treating metabolic disorders involving altered thermogenic responses.

Table 1. Comparison of EC_{50} Values from Lipolysis and Thermogenesis

	EC_{50} (nM)	
	Lipolysis	Thermogenesis
Oligomycin	—	9.2
Rotenone	—	8.5
RO363	1000	956
Albuterol	47.8	43.2
Isoproterenol	27.1	28.0
CL316243	7.9	8.9

ACKNOWLEDGMENTS

The authors would like to thank Lisa Estey for the gift of the yeast strain W303, obtained from the Michael Douglas' laboratory.

REFERENCES

1. J. Himms-Hagen. Role of brown adipose tissue thermogenesis in control of thermoregulatory feeding in rats: a new hypothesis that links thermostatic and glucostatic hypotheses for control of food intake. *Proc. Soc. Exp. Biol. Med.* **208**:159–169 (1995).
2. D. Ricquier, L. Casteilla, and F. Bouillaud. Molecular studies of the uncoupling protein. *FASEB J.* **5**:2237–2242 (1991).
3. C. Fleury, M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi-Meyrueis, F. Bouillaud, M. F. Seldin, R. S. Surwit, D. Ricquier, and C. H. Warden. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* **15**:269–272 (1997).
4. S. Enerback, A. Jacobsson, E. M. Simpson, C. Guerra, H. Yamashita, M. E. Harper, and L. P. Kozak. Mice lacking mitochondrial uncoupling protein are cold- sensitive but not obese. *Nature* **387**:90–94 (1997).
5. M. Shimabukuro, Y. T. Zhou, Y. Lee, and R. H. Unger. Induction of uncoupling protein-2 mRNA by troglitazone in the pancreatic islets of Zucker diabetic fatty rats. *Biochem. Biophys. Res. Commun.* **237**:359–361 (1997).
6. E. E. Blaak, W. H. Saris, and M. A. van Baak. Adrenoceptor subtypes mediating catecholamine-induced thermogenesis in man. *Int. J. Obes. Relat. Metab. Disord.* **17 Suppl** 3:S78–S81 (1993).
7. S. Rehnmark, M. Nechad, D. Herron, B. Cannon, and J. Nedergaard, Alpha-and beta-adrenergic induction of the expression of

the uncoupling protein thermogenin in brown adipocytes differentiated in culture. *J. Biol. Chem.* **265**:16464–16471 (1990).
8. M. Lafontan and M. Berlan. Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* **34**:1057–1091 (1993).
9. J. E. Silva. Full expression of uncoupling protein gene requires the concurrence of norepinephrine and triiodothyronine. *Mol. Endocrinol.* **2**:706–713 (1988).
10. S. Rehnmark, J. Kopecky, A. Jacobsson, M. Nechad, D. Herron, B. D. Nelson, M. J. Obregon, J. Nedergaard, and B. Cannon. Brown adipocytes differentiated in vitro can express the gene for the uncoupling protein thermogenin: effects of hypothyroidism and norepinephrine. *Exp. Cell Res.* **182**:75–83 (1989).
11. O. Champigny and D. Ricquier. Evidence from *in vitro* differentiating cells that adrenoceptor agonists can increase uncoupling protein mRNA level in adipocytes of adult humans: an RT-PCR study. *J. Lipid Res.* **37**:1907–1914 (1996).
12. J. Nedergaard and B. Cannon. [^{3}H]GDP binding and thermogenin amount in brown adipose tissue mitochondria from cold-exposed rats. *Am. J. Physiol.* **248(3 Pt 1)**:C365–C371 (1985).
13. M. Reers, T. W. Smith, and L. B. Chen. J-aggregate formation of a carbocyanine as a quantitative fluorescent indicator of membrane potential. *Biochemistry* **30**:4480–4486 (1991).
14. H. Handesman. Thermography for indications other than breast lesions. *Health Technol. Assess Rep.* **2**:1–32 (1989).
15. W. J. Yang and P. P. Yang. Literature survey on biomedical applications of thermography. *Biomed Mater. Eng.* **2**:7–18 (1992).
16. J. M. Lenhard, S. A. Kliewer, M. A. Paulik, K. D. Plunket, J. M. Lehmann, and J. E. Weiel. Effects of troglitazone and metformin on glucose and lipid metabolism: alterations of two distinct molecular pathways. *Biochem. Pharmacol.* **54**:801–808 (1997).
17. M. A. Paulik and J. M. Lenhard. Thiazolidinediones inhibit alkaline phosphatase activity while increasing expression of uncoupling protein, deiodinase, and increasing mitochondrial mass in C3H10T1/2 cells. *Cell Tissue Res.* **290**:79–87 (1997).
18. S. A. Ahmad-Junan, P. C. Amos, G. S. Cockerill, P. C. Levett, and D. A. Whiting. Synthetic studies on electron transport inhibitors related to natural products. *Biochem. Soc. Trans.* **22**:237–241 (1994).
19. H. Terada. The interaction of highly active uncouplers with mitochondria. *Biochim. Biophys. Acta* **639**:225–242 (1981).
20. J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPAR γ): *J. Biol. Chem.* **270**:12953–12956 (1995).
21. T. A. C. Tai, C. Jennermann, K. K. Brown, B. B. Oliver, M. A. MacGinnitie, W. O. Wilkison, H. R. Brown, J. M. Lehmann, S. A. Kliewer, D. C. Morris, and R. A. Graves. Activation of the nuclear receptor peroxisome proliferator-activated receptor gamma promotes brown adipocyte differentiation. *J. Biol. Chem.* **271**:29909–29914 (1996).
22. P. J. Scarpace. Thermoregulation with age: role of beta-adrenergic signal transduction. *Ann. N. Y. Acad. Sci.* **813**:111–116 (1997).
23. H. Bottcher and P. Furst. A new microcatheter enables microcalorimetric assessment of thermogenesis after stimulation in cell cultures. *J. Biochem. Biophys. Methods* **32**:191–194 (1996).