J. Biochem. 119, 120-125 (1996)

Expression of β3-Adrenoceptor and Stimulation of Glucose Transport by β3-Agonists in Brown Adipocyte Primary Culture[1]

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Received for publication, August 28, 1995

Precursor cells of brown adipocytes were isolated from the interscapular brown fat of newborn rats and cultured on collagen-coated dishes. When confluent cells were treated with dexamethasone, mRNAs for muscle/adipocyte type of glucose transporter, hormone-sensitive lipase, and CCAAT/enhancer binding protein α were increased remarkably, confirming a predominant effect of dexamethasone on the terminal differentiation of the cultured cells. Effects of dexamethasone on the expression of three subtypes of β-adrenoceptor were also examined. β1- and β2-adrenoceptor mRNAs remained constant regardless of dexamethasone-treatment, while β3-adrenoceptor mRNA was present only in dexamethasone-treated differentiated cells. To assess the metabolic response mediated by β3-adrenoceptor, glucose transport into the cells was estimated. Norepinephrine enhanced glucose transport in dexamethasone-treated differentiated cells, but not in undifferentiated cells. β3-Adrenergic agonists mimicked completely the stimulatory effect of norepinephrine at concentrations lower by two orders of magnitude. These results suggest that the β3-adrenoceptor is expressed during the course of differentiation in brown adipocytes and plays a significant role in the response of glucose transport to adrenergic stimulation.

Key words: β-adrenoceptor, β3-agonist, brown adipocyte, glucose transport, norepinephrine.

Brown adipose tissue (BAT) functions for metabolic heat production in response to cold exposure and overfeeding. The metabolic activation and subsequent heat production in BAT is primarily controlled by the sympathetic nerves distributed abundantly to this tissue (*1, 2*). Although the main substrate for BAT thermogenesis is fatty acids derived from intracellular triglyceride, glucose utilization in BAT is also activated by the sympathetic nerves in parallel with heat production (*3-6*). We (*6-9*) have demonstrated in rats that sympathetic stimulation of glucose uptake into BAT is attributable to an increased *de novo* synthesis of muscle/adipocyte type glucose transporter (GLUT4) as well as an increase in the intrinsic activity of the transporter. Most of the stimulatory effects of the sympathetic nerves on BAT glucose uptake are based on the β-adrenergic action of norepinephrine. In brown adipocytes, there are three isoforms of β-adrenoceptor: β1, β2, and β3. Of these, interest has recently focused mostly on the β3-adrenoceptor, because this isoform is expressed primarily, but not exclusively, in brown and white adipocytes (*10-13*). In fact, there are reports that administration of some agonists specific to the β3-adrenoceptor can mimic the action of norepinephrine *in vivo*, enhancing glucose uptake and GLUT4 expression in BAT (*14-17*). However, little is known about the cellular mechanism of glucose transport stimulated by the β3-adrenoceptor pathway and/or its contribution to the β-adrenergic action of norepinephrine.

Recently, we (*18*) have developed the primary culture of rat brown adipocytes, which differentiate only after treatment with dexamethasone to accumulate lipid droplets and express GLUT4. Because their glucose transport is responsive to norepinephrine, these cells are expected to be suitable for studying the mechanism underlying the enhancement of glucose transport induced by norepinephrine and β3-adrenoceptor agonists. In this study, we used this brown adipocyte primary culture to investigate differentiation-dependent changes in expression of the three isoforms of β-adrenoceptor, with special reference to the β3-adrenoceptor, and in the response of glucose transport to norepinephrine and β3-agonists.

EXPERIMENTAL PROCEDURES

Materials—Insulin, dexamethasone, triiodothyronine,

[1] This work was supported by grants from the Mitsubishi Foundation and Uehara Memorial Foundation, and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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Abbreviations: BAT, brown adipose tissue; C/EBP, CCAAT/enhancer binding protein; dGlc, 2-deoxy-D-glucose; GLUT4, muscle/adipocyte type of glucose transporter; RT-PCR, reverse transcriptase-polymerase chain reaction.

collagenase (type I), and BSA were obtained from Sigma. Dulbecco's modified Eagle's medium was from Nissui Pharmaceutical, and fetal calf serum from Sanko Junyaku. M-MLV reverse transcriptase and *Taq* DNA polymerase were from Gibco BRL. A nylon membrane (HybondN$^+$), α-[^{32}P]dCTP and multiprime DNA labeling system were from Amersham. 2-Deoxy-D-[^{3}H]glucose ([^{3}H]dGlc) and [^{14}C]sucrose were from American Radiolabeled Chemicals. cDNA for GLUT4 was kindly given by Dr. G.I. Bell, University of Chicago, and cDNA for CCAAT/enhancer binding protein (C/EBP)β by Dr. S.L. McKnight, Tularik. cDNAs for β-adrenoceptors, hormone-sensitive lipase, C/EBPα, and C/EBPδ were synthesized by the PCR method. β3-Adrenoceptor agonists CL 316,243 and BRL 37344 were provided by American Cyanamid and SmithKline Beecham, respectively.

Cell Isolation and Culture—Brown fat precursor cells were isolated as the stromal-vascular fraction from the interscapular BAT of newborn rats by the procedure described previously (*18*). Briefly, cells isolated by collagenase digestion were suspended in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 17 μM D-pantothenic acid, 33 μM *d*-biotin, 100 μM ascorbic acid, 1 μM octanoic acid, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 50 nM insulin, and 50 nM triiodothyronine, then seeded on collagen-coated dishes. The cells were incubated at 37°C in an atmosphere of 5% CO_2 in air. Where indicated, cultured cells were treated with 1 μM dexamethasone for 48 h when they reached confluence.

RNA Isolation and Northern Blotting—Total RNA was extracted from the cultured cells by the acid guanidinium-phenol-chloroform method (*19*). Poly(A) RNA was prepared using an oligo(dT) cellulose column. Forty micrograms of total RNA or 10 μg of poly(A) RNA was separated on a 1% agarose/formaldehyde gel, then transferred to and fixed on a nylon membrane. The cDNA probes used were: rat GLUT4 cDNA corresponding to nucleotides +1 to +1330 of the published cDNA sequence; rat hormone-sensitive lipase cDNA, +1903 to +2490; rat C/EBPα cDNA, +586 to +865; mouse C/EBPβ cDNA, full-length; rat C/EBPδ cDNA, +76 to +453; rat β1-adrenoceptor cDNA, +1 to +600; mouse β2-adrenoceptor, −168 to +160; and rat β3-adrenoceptor, −4 to +308. Each cDNA probe was labeled with α-[^{32}P]dCTP by the multiprime DNA labeling system. The blots were hybridized to the probes, and the radioactivity was detected using a Bioimage analyzer BAS-1000 (Fuji Film).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)—RT-PCR was used for the detection of β3-adrenoceptor mRNA. The sense and antisense primers for β3-adrenoceptor were 5′-CACCTCGAGATGGCTCCGTGGCCTCAC-3′ and 5′-GTCGGTACCCAAGGGCCAGTGGCCAGTCAGCG-3′, respectively. RNA (1 μg) was annealed to the antisense primer and reverse-transcribed at 42°C for 15 min, then incubated at 99°C for 5 min to denature the enzyme. After addition of the sense primer and *Taq* DNA polymerase, PCR was initiated (94°C for 30 s, 60°C for 30 s, and 72°C for 1.5 min; 40 cycles). The products of PCR were visualized by ethidium bromide staining after agarose gel electrophoresis.

Glucose Transport Measurements—dGlc uptake was measured in differentiated brown adipocytes. Cells seeded in 35-mm dishes were pre-incubated with Dulbecco's modified Eagle's medium for 3 h at 37°C, and treated with adrenoceptor agonists, norepinephrine, BRL 37344 or CL 316,243 during the last 1 h of incubation where indicated. Then, the cells were rinsed twice with a glucose-free HEPES-buffered saline solution (140 mM NaCl, 1 mM $CaCl_2$, 5 mM KCl, 2.5 mM $MgSO_4$, and 20 mM HEPES, pH 7.4). After incubation with HEPES-buffered saline containing 2% fatty acid-free BSA in the presence or absence of the agonists for 20 min, the cells were further incubated with 50 μM [^{3}H]dGlc (370 Bq/nmol) in the same solution. Transport was allowed to proceed for 2 min and was terminated by rapid suction and subsequent washing with ice-cold 3 mM $HgCl_2$ in phosphate-buffered saline. Contamination of the isotope due to the extracellular space was corrected using [^{14}C]sucrose. The net uptake of dGlc was expressed in pmol/min/mg protein ± SE.

RESULTS

Effect of Dexamethasone on the mRNA Levels of GLUT4, Hormone-Sensitive Lipase, and C/EBPs in Cultured Brown Adipocytes—We recently showed morphologically and biochemically that dexamethasone accelerates brown adipocyte differentiation in culture (*18*). To confirm the predominant effect of dexamethasone on the terminal differentiation of the cells, the mRNA levels of GLUT4, hormone-sensitive lipase, and C/EBPα, whose expression in adipocytes is known to occur in the late stages of differentiation (*20*), were measured by Northern blot analysis. In proliferating cells as well as cells which had reached confluence, these mRNAs were almost undetectable (Fig. 1), indicating that the cell population at the start of culture was made up largely of undifferentiated precursor cells without contamination of mature brown adipocytes. As expected, mRNAs of GLUT4, hormone-sensitive lipase and C/EBPα increased remarkably after the treatment with dexamethasone (Fig. 1, A-C). In contrast, they were not detected in time-matched, dexamethasone-untreated cells, which are hereafter referred as control cells (Fig. 1, A-C). These results indicate that our differentiation protocol with dexamethasone provides appropriate experimental conditions for the assessment of differentiation-dependent changes in brown adipocytes. Cao *et al.* reported in 3T3 cells that not only C/EBPα but also other two isoforms, C/EBPβ and δ, participate in the regulation of adipose differentiation (*21*). To see if these transcription factors contribute to dexamethasone-induced maturation of brown adipocytes, we also measured mRNA levels of C/EBPβ and C/EBPδ. In contrast to C/EBPα mRNA, the mRNAs of C/EBPβ and δ were present in both confluent and control cells, being at similar levels to the dexamethasone-treated cells (Fig. 1, C-E).

Effect of Dexamethasone on the Expression of β-Adrenoceptors—The mRNA level of β-adrenoceptors was measured by Northern blotting. We first tried to analyze the mRNA of β-adrenoceptors by rehybridization of the membranes used in Fig. 1, to which 40 μg total RNA was blotted. However, no signal was detected under our experimental conditions, probably because of low expression of these receptors. Next, 10 μg of poly(A) RNA was purified from the total RNA and subjected to the analysis. As shown in Fig. 2B, significant levels of mRNA for β1- and β2-adrenoceptors were found in confluent cells, while β3-adreno-

ceptor mRNA was not detected. After the dexamethasone-treatment, mRNA levels for β1- and β2-adrenoceptors remained constant, but a strong signal of β3-adrenoceptor mRNA appeared (Fig. 2B). The relative level of each mRNA in dexamethasone-treated cells was similar to those in the interscapular BAT, being highest for β3-adrenoceptor, high for β1-adrenoceptor and low for β2-adrenoceptor (Fig. 2A). To see if the absence of signal for β3-adrenoceptor mRNA in confluent cells was due to the limited sensitivity of Northern blot analysis, we applied the RT-PCR method for detection of β3-adrenoceptor mRNA in the cells. In dexamethasone-treated cells as well as the tissue, clear bands were seen after staining with ethidium bromide (Fig. 3). A putative 324-bp PCR product of β3-adrenoceptor mRNA was isolated, sequenced using a DNA sequencer (ABI 373A DNA sequencer) and confirmed as the true product. In contrast, no band was found in confluent and control cells. Thus, β3-adrenoceptor was expressed in the primary cultured brown adipocytes only after the dexamethasone-treatment.

Effects of Norepinephrine, BRL 37344, and CL 316,243 on dGlc Uptake of Cultured Brown Adipocytes—It has been shown that primarily cultured brown adipocytes increase glucose transport in response to norepinephrine through the β-adrenergic pathway, as do freshly isolated brown adipocytes. To assess the functional response through the β3-adrenoceptor in the cultured adipocytes, effects of norepinephrine and β3-adrenoceptor agonists on glucose transport were examined by measuring the cellular uptake of [^{3}H]dGlc. In control cells, neither norepinephrine nor β3-agonists (BRL 37344 and CL 316,243) influenced [^{3}H]-dGlc uptake (Fig. 4). However, in dexamethasone-treated cells, β3-agonists as well as norepinephrine effectively stimulated [^{3}H]dGlc uptake, indicating the existence of functionally active β3-adrenoceptor in differentiated brown adipocytes in culture. Figure 5 shows dose-response curves for the action of norepinephrine and CL 316,243.

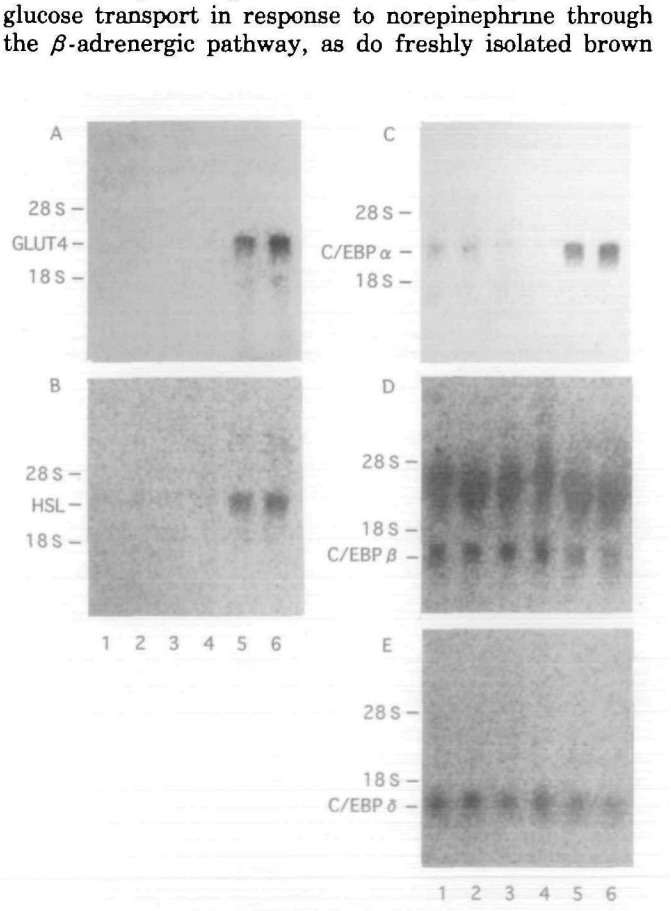

Fig. 1 Effect of dexamethasone on the expression of GLUT4, hormone-sensitive lipase, C/EBPα, C/EBPβ, and C/EBPδ in cultured brown adipocytes. When cultured brown adipocytes reached confluence, they were cultured for 2 days in the presence or absence (control) of 1 μM dexamethasone Total RNA was isolated from confluent (lanes 1 and 2), control (lanes 3 and 4), and dexamethasone-treated (lanes 5 and 6) cells Samples of 40 μg were subjected to Northern blot analysis A, GLUT4, B, hormone-sensitive lipase (HSL); C, C/EBPα, D, C/EBPβ; E, C/EBPδ.

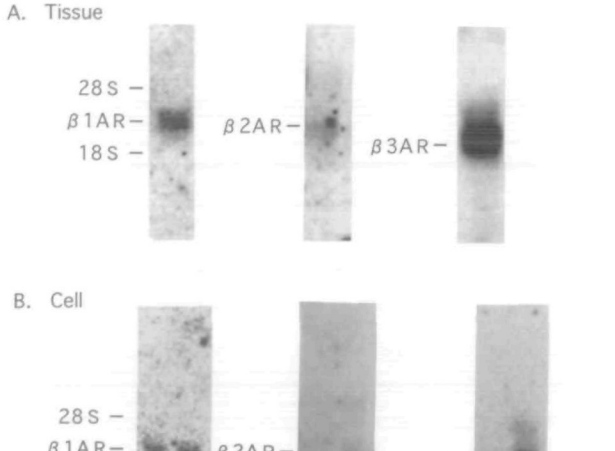

Fig. 2 Effect of dexamethasone on the expression of β-adrenoceptors mRNA in cultured brown adipocytes. A Poly(A) RNA was purified from total RNA of rat BAT, and samples of 10 μg were subjected to Northern blot analysis B: Poly(A) RNA was purified from total RNA of confluent (lane 1) and dexamethasone-treated (lane 2) cells, and samples of 10 μg were subjected to Northern blot analysis AR, adrenoceptor

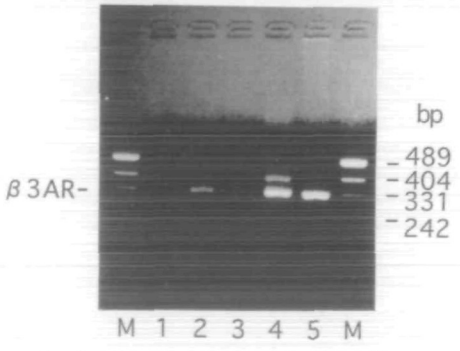

Fig. 3. **Analysis of β3-adrenoceptor mRNA with RT-PCR.** Total RNA (250 ng) was reverse-transcribed and subjected to PCR. After agarose gel electrophoresis, PCR products were stained with ethidium bromide. Lane 1, confluent cells, lane 2, dexamethasone-treated cells, lane 3, control cells; lane 4, rat BAT; lane 5, PCR product from 250 ng of rat genomic DNA; lane M, size marker AR, adrenoceptor.

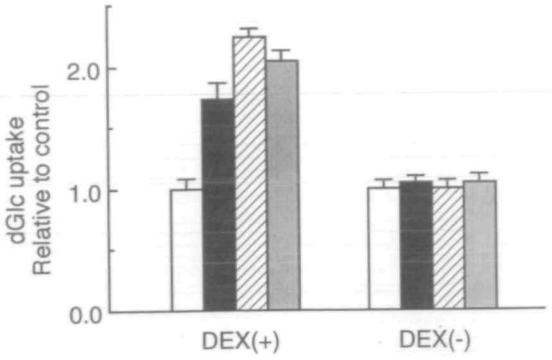

Fig. 4. **Effect of norepinephrine, BRL 37344, and CL 316,243 on dGlc uptake into cultured brown adipocytes.** The uptake of dGlc by cultured brown adipocytes was measured in the absence (□) or presence of 10^{-7} M norepinephrine (■), 10^{-7} M BRL 37344 (▨), and 10^{-7} M CL 316,243 (▣). Values are means±SE of four independent experiments. The mean uptake in controls was 4.44±0.36 pmol/min/mg protein.

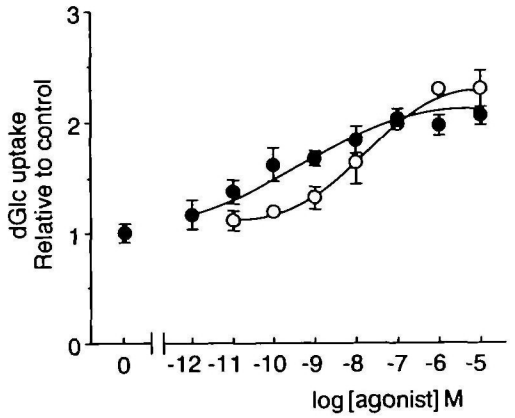

Fig. 5. **Dose-response curves for norepinephrine and CL 316,243 on dGlc uptake into cultured brown adipocytes.** The uptake of dGlc was measured at various concentrations of norepinephrine (○) or CL 316,243 (●). Values are means±SE of three experiments.

Norepinephrine increased [^{3}H]dGlc uptake progressively at concentrations from 10^{-11}–10^{-6} M with a maximal effect at 10^{-6} M. CL 316,243 also increased [^{3}H]dGlc uptake in a similar manner to norepinephrine. The maximum rate of [^{3}H]dGlc uptake by CL 316,243 was comparable with that by norepinephrine, but the dose-response curve was shifted to the left to concentrations lower by about two orders lower of magnitude than norepinephrine.

DISCUSSION

In the present study, first, we confirmed the promoting effect of dexamethasone on the terminal differentiation of brown adipocytes in primary culture, as judged by the expression of several marker genes for adipocyte differentiation including GLUT4, hormone-sensitive lipase and C/EBPα. Next, the mRNA levels of β-adrenoceptors in the differentiated cells were compared with those in undifferentiated control cells to explore the differentiation-dependent changes in the expression of β-adrenoceptor subtypes, especially of β3-adrenoceptor. Our results showed that: (i) β3-adrenoceptor became detectable only after treating the cells with dexamethasone, suggesting β3-adrenoceptor expression is dependent on the differentiation process—in contrast, the expression of β1- and β2-adrenoceptors is little affected during the course of proliferation and differentiation; and (ii) a brown fat-specific metabolic response to β3-agonists, *i.e.*, an increase in glucose transport, parallels the emergence of β3-adrenoceptor.

β3-Adrenoceptor mRNA was found in high level in the interscapular BAT, whereas it was detected only in differentiated brown adipocytes, not in preadipocytes, in culture. It is thus likely that β3-adrenoceptor is expressed *in situ* in matured adipocytes of the tissue. Our finding of differentiation-dependent expression of β3-adrenoceptor in brown adipocytes is consistent with the results in 3T3-F442A cells, which also express β3-adrenoceptor only after differentiation. However, it should be noted that 3T3-F442A cells differentiate in the absence of glucocorticoids (*22*). Moreover, in contrast to the brown adipocytes, dexamethasone suppressed β3-adrenoceptor expression in 3T3-F442A cells (*23*). In support of these *in vitro* observations, adrenalectomy was reported to increase β3-adrenoceptor mRNA in BAT of obese Zucker rats (*24*). Thus, the suppression of β3-adrenoceptor expression seems to be a direct effect of glucocorticoid on the gene transcription in mature adipocytes. Indeed, there are recognition sequences of the glucocorticoid receptor in the 5′ flanking region of β3-adrenoceptor gene, and negative interaction with a transcription factor AP-1 or adipose-specific factors is proposed (*25*). Beside such a direct effect of glucocorticoid on gene expression, the hormone is known to trigger the sequential events for adipose conversion of pre-adipocytes (*20*). This was also the case in the cultured brown adipocytes: that is, dexamethasone induced a notable accumulation of lipid droplets and expression of GLUT4 and hormone-sensitive lipase, in addition to an increased expression of a transcription factor, C/EBPα, which is associated with the differentiation process of adipocytes (Fig. 1, and Refs. *18, 21,* and *26*). Collectively, it seems reasonable to assume that induction of β3-adrenoceptor after the dexamethasone treatment in brown adipocytes is secondary to cell maturation, not due to a direct effect of the hormone. This assumption may explain the apparently opposite effects of dexamethasone on β3-adrenoceptor expression in these two cells as a difference in the stage of differentiation rather than one of cell type.

It has been demonstrated *in vivo* and *in vitro* that glucose utilization in brown adipocytes is stimulated not only by insulin but also norepinephrine (*3, 6, 18, 27*). The stimulatory action of norepinephrine is BAT-specific (*3, 6*) and is mediated through the β-adrenergic pathway. In the present study, we found that β3-agonists CL 316,243 and BRL 37344 mimicked the action of norepinephrine to enhance [^{3}H]dGlc uptake in cultured brown adipocytes, in parallel with the emergence of β3-adrenoceptor. The maximal response to CL 316,243 was almost identical to that to norepinephrine, but its effective concentrations were about two orders of magnitude lower than those of norepinephrine, being consistent with the difference in the binding affinity of the two agonists for the rat β3-adrenoceptor (*28, 29*). These results suggest that norepinephrine-induced increase in glucose transport is mediated largely through β3-adrenoceptor. The importance of β3-adrenoceptor in

the acute metabolic effect of norepinephrine on adipocytes was also proposed in the responses of lipolysis and cAMP accumulation (*10*). The stimulatory effect of the β3-agonist was observed only in the differentiated cells expressing β3-adrenoceptor, but not in the undifferentiated control cells although these cells expressed β1- and β2-adrenoceptors. This may provide an additional support for a significant role of β3-adrenoceptor in activating glucose transport. It is to be noted, however, that the parallel appearance of β3-adrenoceptor and β3-agonist-induced increase in glucose transport does not necessarily mean that the presence of β3-adrenoceptor itself is sufficient for the response of glucose uptake to β3-agonists and norepinephrine. In fact, direct stimulation of the pathways beyond the β-adrenoceptors by a membrane-permeable cAMP analogue, which can mimic the catecholamine-induced increase in glucose transport in the differentiated cells, did not enhance glucose uptake in undifferentiated control cells (unpublished observation). Undoubtedly, full activation of glucose uptake by the adrenergic agonists is based on differentiation-dependent expression of not only β3-adrenoceptor but also many post-receptor components such as GLUT4.

The present finding of β3-adrenoceptor expression only in differentiated brown adipocytes suggests a likely explanation for some apparently discrepant results on the effects of adrenergic agonists so far reported. For example, administration of norepinephrine *in vivo* stimulates the expression of some specific genes, and proliferation and maturation of preadipocytes, leading to a marked hyperplasia of BAT (*9*). Administration of β3-agonist can mimic in part the effects of norepinephrine but does not stimulate cell proliferation, and thus results in hypertrophy of the tissue (*30*). In accordance with these *in vivo* observations, Bronnikov *et al.* reported that the proliferation of preadipocytes by norepinephrine is brought about by the mediation *via* β1-adrenoceptor but not *via* β3-adrenoceptor (*31*).

Several lines of evidence allow us to assess the physiological significance of specific expression of β3-adrenoceptor in BAT. One of the properties of β3-adrenoceptor is its low affinity for catecholamines compared with β1- and β2-adrenoceptors (*10-13*). This might eliminate the effective contribution of circulating catecholamines to BAT thermogenesis, permitting the exclusive stimulation by norepinephrine locally released from sympathetic nerves. Moreover, β3-adrenoceptor has no consensus sequence for phosphorylation by cAMP-dependent protein kinase and few by β-adrenoceptor kinase (*32-34*), suggesting that β3-adrenoceptor is highly resistant to desensitization. This might be a beneficial feature for BAT, because continuous and prolonged activation of thermogenesis is required in order to maintain body temperature in a cold environment. Thus, the emergence of β3-adrenoceptor upon differentiation seems to be an essential component for the functional maturation of brown adipocytes.

We are grateful to Dr. G.I. Bell (Univ. Chicago) for providing rat cDNA probe for GLUT4, Dr. S.L. McKnight (Tularik) for mouse cDNA for C/EBPβ, Dr. M.A. Cawthorne (SmithKline Beecham) for the BRL 37344, and American Cyanamid for CL 316,243.

REFERENCES

1. Nicholls, D.G. and Locke, R.M. (1984) Thermogenic mechanisms in brown fat. *Physiol. Rev.* **64**, 1-64
2. Girardier, L. and Sedoux, J. (1986) Neural control of brown adipose tissue in *Brown Adipose Tissue* (Thrayhun, P. and Nicholls, D., eds.) pp. 122-151, Edward Arnold, London
3. Cooney, G.J., Caterson, I.D., and Newsholme, E.A. (1985) The effect of insulin and noradrenaline on the uptake of 2-[1-^{14}C]-deoxyglucose *in vivo* by brown adipose tissue and other glucose-utilising tissue of the mouse. *FEBS Lett.* **188**, 257-261
4. Greco-Perotto, R., Zaninetti, D., Assimacopoulos-Jannet, F., Bobbioni, E., and Jeanrenaud, B. (1987) Stimulatory effect of cold adaptation on glucose utilization by brown adipose tissue. *J. Biol. Chem.* **262**, 7732-7736
5. Vallerand, A., Perusse, F., and Bukowiecki, L.J. (1990) Stimulatory effects of cold exposure and cold acclimation on glucose uptake in rat peripheral tissues. *Am. J. Physiol.* **259**, R1043-R1049
6. Shimizu, Y., Nikami, H., and Saito, M. (1991) Sympathetic activation of glucose utilization in brown adipose tissue in rats. *J. Biochem.* **110**, 688-692
7. Nikami, H., Shimizu, Y., Endoh, D., Yano, H., and Saito, M. (1992) Cold exposure increases glucose utilization and glucose transporter expression in brown adipose tissue. *Biochem. Biophys. Res. Commun.* **185**, 1078-1082
8. Shimizu, Y., Nikami, H., Tsukazaki, K., Machado, U.F., Yano, H., Seino, Y., and Saito, M. (1993) Increased expression of glucose transporter GLUT-4 in brown adipose tissue of fasted rats after cold exposure. *Am. J. Physiol.* **264**, E890-E895
9. Tsukazaki, K., Nikami, H., Shimizu, Y., Kawada, T., Yoshida, T., and Saito, M. (1995) Chronic administration of β-adrenergic agonists can mimic the stimulative effect of cold exposure on protein synthesis in rat brown adipose tissue. *J. Biochem.* **117**, 96-100
10. Arch, J.R.S. and Kaumann, A.J. (1993) β3 and atypical β-adrenoceptors. *Med. Res. Rev.* **13**, 663-729
11. Lafontan, M. and Berlan, M. (1993) Fat cell adrenergic receptors and control of white and brown fat cell function. *J. Lipid Res.* **34**, 1057-1091
12. Giacobino, J.P. (1995) β3-Adrenoceptor: An update. *Eur. J. Endocrinol.* **132**, 377-385
13. Granneman, J.G., Lahners, K.N., and Chaudhry, A. (1992) Molecular cloning and expression of the rat β3-adrenergic receptor. *Mol. Pharmacol.* **40**, 895-899
14. Le Marchand-Brustel, Y., Olichon-Berthe, C., Gremeaux, T., Tanti, J.F., Rochet, N., and Van Obberghen, E. (1990) Glucose transporters in insulin sensitive tissues of lean and obese mice. Effects of the thermogenic agent BRL 26830A. *Endocrinology* **127**, 2687-2695
15. Assimacopoulos-Jeannet, F., Greco-Perotto, R., Terrettaz, J., Meier, M.K., and Jeanrenaud, B. (1992) Effect of a β-adrenergic agonist on glucose transport and insulin-responsive glucose-transporters (GLUT4) in brown adipose tissue of control and obese *fa/fa* rats. *Pflugers Arch.-Eur. J. Physiol.* **421**, 52-58
16. Nagase, I., Sasaki, N., Tsukazaki, K., Yoshida, T., Morimatsu, M., and Saito, M. (1994) Hyperplasia of brown adipose tissue after chronic stimulation of β3-adrenergic receptor in rats. *Jpn. J. Vet. Res.* **42**, 137-145
17. Liu, Y.L. and Stock, M.J. (1995) Acute effects of the β3-adrenoceptor agonist, BRL 35135, on tissue glucose utilization. *Br. J. Pharmacol.* **114**, 888-894
18. Shimizu, Y., Kielar, D., Masuno, H., Minokoshi, Y., and Shimazu, T. (1994) Dexamethasone induces the GLUT4 glucose transporter, and responses of glucose transport to norepinephrine and insulin in primary cultures of brown adipocytes. *J. Biochem.* **115**, 1069-1074
19. Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156-159
20. Aihaud, G., Grimaldi, P., and Negrel, R. (1992) Cellular and molecular aspects of adipose tissue development. *Annu. Rev. Nutr.* **12**, 207-233
21. Cao, Z., Umek, R.M., and McKnight, S.L. (1991) Regulated expression of three C/EBP isoforms during adipose conversion of

3T3-L1 cells. *Genes Dev.* **5**, 1538-1552
22. Feve, B., Emorine, L.J., Lasnier, F., Blin, N., Baude, B., Nahmias, C., Strosberg, A.D., and Pairault, J. (1991) Atypical β-adrenergic receptor in 3T3-F442A adipocytes. Pharmacological and molecular relationship with the human β3-adrenergic receptor. *J. Biol. Chem.* **266**, 20329-20336
23. Feve, B., Baude, B., Krief, S., Strosberg, A.D., Pairault, J., and Emorine, L.J. (1992) Inhibition by dexamethasone of β3-adrenergic receptor responsiveness in 3T3-F442A adipocytes. Evidence for a transcriptional mechanism. *J. Biol. Chem.* **267**, 15909-15915
24. Okada, S., Onai, T., Kilroy, G., York, D.A., and Bray, G.A. (1993) Adrenalectomy of the obese Zucker rat—Effects on the feeding response to enterostatin and specific messenger RNA levels. *Am. J. Physiol.* **265**, R21-R27
25. Van Spronsen, A., Nahmias, C., Krief, S., Briend Sutren, M.M., Strosberg, A.D., and Emorine, L.J. (1993) The promoter and intron/exon structure of the human and mouse β3-adrenergic-receptor genes. *Eur. J. Biochem.* **213**, 1117-1124
26. Shima, A., Shinohara, Y., Doi, K., and Terada, H. (1994) Normal differentiation of rat brown adipocytes in primary culture judged by their expressions of uncoupling protein and the physiological isoform of glucose transporter. *Biochim. Biophys. Acta* **1223**, 1-8
27. Marette, A. and Bukowiecki, L.J. (1991) Noradrenaline stimulates glucose transport in rat brown adipocytes by activating thermogenesis. Evidence that fatty acid activation of mitocondrial respiration enhances glucose transport. *Biochem. J.* **277**, 119-124
28. Emorine, L., Blin, N., and Strosberg, A.D. (1994) The human β3-adrenoceptor: The search for a physiological function. *Trends Pharmacol. Sci.* **15**, 3-7
29. D'Allaire, F., Atgie, C., Mauriege, P., Simard, P.M., and Bukowiecki, L.J. (1995) Characterization of β1- and β3-adrenoceptors in intact brown adipocytes of the rat. *Br. J. Pharmacol.* **114**, 275-282
30. Himms-Hagen, J., Cui, J., Danforth, E., Jr., Taatjes, D.J., Lang, S.S., Waters, B.L., and Claus, T.H. (1994) Effect of CL-316,243, a thermogenic β3-agonist, on energy balance and brown and white adipose tissues in rats. *Am. J. Physiol.* **266**, R1371-R1382
31. Bronnikov, G., Houstek, J., and Nedergaard, J. (1992) β-Adrenergic, cAMP-mediated stimulation of proliferation of brown fat cells in primary culture. Mediation via β1 but not via β3 adrenoceptors. *J. Biol. Chem.* **267**, 2006-2013
32. Hausdorff, W.P., Caron, M.G., and Lefkowitz, R.J. (1990) Turning off the signal: Desensitization of β-adrenergic receptor function. *FASEB J.* **4**, 2881-2889
33. Nantel, F., Bonin, H., Emorine, L.J., Zilberfarb, V., Storosberg, A.D., Bouvier, M., and Marullo, S. (1993) The human β3-adrenergic receptor is resistant to short term agonist-promoted desensitization. *Mol. Pharmacol.* **43**, 548-555
34. Liggett, S.B., Freedman, N.J., Schwinn, D.A., and Lefkowitz, R.J. (1993) Structural basis for receptor subtype-specific regulation revealed by a chimeric β3/β2-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **90**, 3665-3669