β3-Adrenergic Receptors on White and Brown Adipocytes Mediate β3-Selective Agonist-induced Effects on Energy Expenditure, Insulin Secretion, and Food Intake

A STUDY USING TRANSGENIC AND GENE KNOCKOUT MICE*

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β3-Adrenergic receptors (β3-ARs) are expressed predominantly on white and brown adipocytes, and acute treatment of mice with CL 316,243, a potent and highly selective β3-AR agonist, produces a 2-fold increase in energy expenditure, a 50–100-fold increase in insulin levels, and a 40–50% reduction in food intake. Recently, we generated gene knockout mice lacking functional β3-ARs and demonstrated that each of these responses were mediated exclusively by β3-ARs. However, the tissue site responsible for producing these actions is unknown. In the present study, genetically engineered mice were created in which β3-ARs are expressed exclusively in white and brown adipocytes (WAT-bat, BAT-mice), or in brown adipocytes only (BAT-mice). This was accomplished by injecting tissue-specific β3-AR transgenic constructs into mouse zygotes homozygous for the β3-AR knockout allele. Control, knockout, WAT+BAT, and BAT-mice were then treated acutely with CL, and the effects on various parameters were assessed. As previously observed, all effects of CL were completely absent in gene knockout mice lacking β3-ARs. The effects on O₂ consumption, insulin secretion, and food intake were completely rescued with transgenic re-expression of β3-ARs in white and brown adipocytes (WAT+BAT-mice), demonstrating that each of these responses is mediated exclusively by β3-ARs in white and/or brown adipocytes, and that β3-ARs in other tissue sites were not required. Importantly, transgenic re-expression of β3-ARs in brown adipocytes only (BAT-mice) failed to rescue, in any way, CL-mediated effects on insulin levels and food intake and only minimally restored effects on oxygen consumption, indicating that any effect on insulin secretion and food intake, and a full stimulation of oxygen consumption required the presence of β3-ARs in white adipocytes. The mechanisms by which β3-AR agonist stimulation of white adipocytes produces these responses are unknown but may involve novel mediators not previously known to effect these processes.

Obesity is a prevalent condition frequently associated with diabetes, hypertension, and cardiovascular disease. Because available treatments are minimally effective, substantial efforts have been directed toward the discovery of new, effective, anti-obesity drugs. The β3-adrenergic receptor (β3-AR) represents one of a number of potential anti-obesity drug targets for which selective agonists have been developed (1–3). The β3-AR is encoded by a distinct gene that is expressed predominantly in white and brown adipocytes (4–7), important sites for energy storage and energy expenditure, respectively. Selective activation of β3-ARs leads to marked increases in triglyceride breakdown (lipolysis) and energy expenditure (1–3), and long-term treatment of obese rodents with β3-selective agonists reduces fat stores and improves obesity-induced insulin resistance (1–3). Thus, β3-selective agonists are promising anti-obesity compounds.

Acute treatment of rodents with β3-selective agonists causes a number of diverse metabolic effects including a 2-fold increase in energy expenditure as measured by effects on oxygen consumption, a 10–100-fold increase in insulin levels, and a 40–50% reduction in food intake (8–11). While the effects on lipolysis and energy expenditure are likely to be mediated by β3-ARs, uncertainty has existed regarding the identity of receptors mediating the acute effects on insulin levels and food intake. Recently, we generated gene knockout mice lacking functional β3-ARs (11). These animals have a slight increase in body fat; but otherwise, they appear to be normal, probably because of adaptations which compensate for the absence of β3-ARs, one example being the observed up-regulation of β1-AR gene expression in brown and white adipose tissue (11). Of significance, these mutant mice are completely resistant to the ability of CL 316,243 (1), a β3-selective agonist, to increase lipolysis, energy expenditure, insulin levels, and to reduce food intake (11). Thus, each of these effects is mediated exclusively by β3-ARs.

The abbreviations used are: β-AR, β-3-adrenergic receptor; BAT-mice, transgenic mice with β3-ARs in brown fat only; CL, CL 316,243; FFA, free fatty acid; UCP, uncoupling protein; WAT+BAT-mice, transgenic mice with β3-ARs in white and brown fat only; bp, base pair(s); kb, kilobase(s); PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR.
The relative role of β3-ARs in white versus brown adipocytes, as well as β3-ARs in other sites, in mediating each of these responses is also unresolved. For example, is the marked stimulatory effect of β3-selective agonists on energy expenditure mediated exclusively by stimulation of brown adipocytes, or must white adipocytes also be stimulated to supply free fatty acids as fuel for brown adipocytes? Furthermore, as β3-ARs have been reported to exist in the gastrointestinal tract, brain, heart, and prostate and since other sites of expression cannot be excluded (5, 12–15), is it possible that receptors in non-adipocyte locations mediate some or all of the effects on energy expenditure, insulin secretion, and food intake? At present, the answers to questions such as these are unknown.

In general, it has been difficult to determine the relative role of various target tissues in mediating complex physiologic responses. Genetic engineering in mice, however, provides a means by which these issues can be addressed. In the present study, we have combined gene knockout and transgenic techniques to create mice in which functional β3-ARs are completely absent (knockout mice; see Ref. 11), or are expressed exclusively in selected tissues, namely white and brown adipose tissue (WAT-BAT-mice) or brown adipose tissue only (BAT-mice). To create WAT+BAT-mice and BAT-mice, transgenic constructs were generated in which murine β3-AR gene expression is driven by the tissue-specific promoter/enhancers, aP2 for white and brown adipose tissue expression (16) and UCP for brown adipose tissue expression (17, 18). These transgenic constructs were then injected into fertilized mouse zygotes homozygous for the β3-AR gene knockout allele (11), thus creating mice in which functional β3-ARs are restricted to white and brown fat (WAT+BAT-mice), or brown fat only (BAT-mice). Control, knockout, WAT+BAT-, and BAT-mice were then used to investigate the relative role of β3-ARs in white versus brown adipose tissue, as well as β3-ARs in other sites, in mediating a number of responses to β3-selective agonists.

**EXPERIMENTAL PROCEDURES**

**β3-AR Transgenes and Zygote Microinjections—**Partial restriction enzyme maps for the murine β3-AR wild-type allele, the β3-AR knockout allele (11), the aP2-β3-AR transgene used to create WAT+BAT-mice, and the UCP-β3-AR transgene used to create BAT-mice are shown in Fig. 1. The gene knockout allele is notable for its absence of 306 bp of β3-AR coding sequence, NheI to XhoI, spanning from β3-AR residue 120 in the middle of the third transmembrane domain to residue 222 in the COOH-terminal end of the fifth transmembrane domain. The aP2-β3-AR transgene was created by fusing 5.4 kb of 5′-flanking regulatory sequence of the murine aP2 gene (16), −5.4 kb (EcoRI) to +21 bp (PstI), to a 3.0-kb fragment of mouse β3-AR genomic sequence (7, 19), spanning from 11 bp 5′ of the β3-AR start codon (NarI) to approximately 240 bp 3′ of the β3-AR AATAAA polyadenylation signal (BglII). The UCP-β3-AR transgene was created by fusing 4.0 kb of 5′-flanking regulatory sequence of the murine UCP gene (17), −3.9 kb (EcoRI) to +120 bp (PstI), to the mouse β3-AR 3.0-kb fragment described above. The transgenes were excised from plasmid vector sequence, gel-purified, and then injected into male pronuclei (20) of zygotes homozygous for the β3-AR knockout allele (11). To identify transgenic animals, genomic DNA from mouse tails was digested with BamHI (aP2-β3-AR construct) or NcoI (UCP-β3-AR construct), electrophoresed, Southern blotted, and hybridized to an 862-bp β3-AR genomic probe (between 545 bp 5′ and 317 bp 3′ of the β3-AR ATG).

β3-AR knockout mice were originally created on an inbred FVB background (11). The knockout allele and the WAT+BAT and BAT-transgenes have been maintained on an inbred FVB background. Control animals were wild-type inbred FVB mice housed under conditions similar to the genetically modified animals. All animals were housed at 24 °C, had free access to food (Purina Chow 5008) and water, and were handled in accordance with the principles and guidelines established by the National Institutes of Health.

**RNA Analyses—**Total RNA was isolated from white and brown adipose tissue, brain, colon, kidney, liver, and skeletal muscle of male mice, age 12 to 20 weeks old, using a Brinkmann homogenizer and RNA STAT-60 solution (Tel-Test “B”, Inc., Friendswood, TX). β3-AR mRNA was detected using standard Northern blotting techniques and 20 μg of total RNA. A β3-AR hybridization probe was generated by random priming from a 306-bp cDNA template corresponding to codons 120–222. Note that this 306-bp cDNA region corresponds to the NheI to XhoI β3-AR fragment deleted during creation of the β3-AR gene knockout allele. The gels were stained with ethidium bromide, and the abundance of 18S and 28S ribosomal bands were used to establish equal loading. To quantitate β3-AR mRNA expression, radioactive signals from all Northern blots were analyzed using a PhosphorImager (Molecular Dynamics, Image Quant software).

**Quantitative Real-Time PCR Analysis of β3-AR mRNA Levels in Isolated Pancreatic Islets—**Five hundred pancreatic islets were isolated from 10 normal mice as described previously (21). Total RNA was extracted using RNA STAT-60 solution and was then subjected to RT-PCR analysis using mouse β3-AR and actin primer sets (β3-AR sense primer, CTCAGCT-GTCAACCACCTT; β3-AR antisense primer, GACGAGAGGATCATCA-CAAAGG; actin sense primer, GTGGGCGGCTGTAGCACAAC; actin antisense primer, CGGTTGCCCTAAGGCTGAGGGG) RNA samples were treated with DNase prior to performing RT-PCR. The β3-AR primer set amplifies a 260-bp fragment, and the actin primer set amplifies a 245-bp fragment. First strand synthesis was performed using the SuperScript preamplification system procedure (Life Technologies, Inc., Gaithersburg, MD) and the antisense primers for β3-AR and actin listed above. PCR was then performed using Takara DNA polymerase. Manufacturers recommended buffer, nucleotide, and primer concentrations were employed. The samples were heated to 94°C for 1.5 min and then cycled for 30 cycles using the following protocol: 98°C for 20 s, 60°C for 1 min, and 68°C for 1.5 min. The PCR products were then analyzed using agarose gel electrophoresis and ethidium bromide staining.

**Effect of CL on Oxygen Consumption—**Oxygen consumption was measured in female, 16–20-week-old control, knockout, WAT+BAT-, and BAT-mice before and after treatment with CL at 1 mg/kg body weight (subcutaneously). Preliminary experiments with control mice indicated that this dose exerted maximum effects on oxygen consumption. Oxygen consumption was measured using computerized equipment that included a 1-liter chamber maintained at 29°C, an air flow of 500 ml/min (regulated with a mass flowmeter, Brooks Instrument Division, Emerson Electric), and an oxygen analyzer (Beckman Industrial Oxygen Analyzer model 755). Mice were awake and unrestrained for the study. The resting rate of oxygen consumption (basal) was assessed when the mice were curred up and still, usually 1–2 h after they had been placed in the chamber. The animals were then injected with CL and returned to the chamber. After injection of CL (1 mg/kg) a constant rate of oxygen uptake was assessed when mice were again resting.

**Effect of CL on Adenylate Cyclase Activity in Membranes Isolated from Brown Adipose Tissue—**Membranes were isolated from interscapular brown adipose tissue of control, knockout, and BAT-mice, and effects of CL on adenylate cyclase activity were assessed as described previously (11).

**Effect of CL on Insulin, Glucose, and Free Fatty Acids (FFAs) Levels—**CL (1 mg/kg body weight, intraperitoneal) or saline was injected into female, 8–12-week-old control, knockout, WAT+BAT-, and BAT-mice; 15 min later, the animals were quickly sacrificed using a small animal decapitator. Whole blood was collected and analyzed for blood glucose levels (One Touch Blood Glucose Meter, LifeScan, Inc., Milpitas, CA). Serum was then isolated and assayed for FFAs (NEFA C Kit, Wako Pure Chemical Industries, Ltd.) and insulin (Insulin Kit, Linco Research Inc., St. Louis, MO). In a preliminary experiment, control mice were injected with CL, and blood samples were obtained before CL and 5, 10, 15, and 60 min after CL injection. Maximal increases in insulin were observed at the 15-min point.

**Effect of CL on Insulin Secretion from Isolated Pancreatic Islets—**Groups of 20 islets from control, knockout, and WAT+BAT-mice were pre-incubated for 30 min in 3 mM glucose as described previously (21). The media was removed and then replaced with 1 ml of the following test solutions: 3 mM glucose, 11 mM glucose, or 11 mM glucose plus 1 μM CL. Of note, 11 mM glucose is a submaximal stimulatory concentration of glucose. Twenty min later, the incubation media was removed and assayed for insulin.

**Effect of CL on Food Intake—**The effect of a single injection of CL (1 mg/kg body weight, intraperitoneal) on food intake was assessed in male, 8–12-week-old control, knockout, WAT+BAT-, and BAT-mice. The mice were housed individually during the study. For three days prior to the day of CL treatment, the mice received daily injections of saline to acclimate them to handling. On the day of study, each experimental group was divided in half, and one half was treated with saline.
while the other half received CL. Food was weighed before and 24 h after injection, and the weight of food missing was assumed to represent g of food eaten. The cages were inspected for food spillage and none was noted.

RESULTS

Generation of Transgenic Mice—A large colony of homozygous β3-AR knockout mice was established (11) and used to generate homozygous β3-AR knockout zygotes. These zygotes were injected with the aP2-β3-AR or UCP-β3-AR transgenes

Fig. 1. The murine β3-AR gene, the targeted β3-AR gene, and the aP2-β3-AR and UCP-β3-AR transgenes. Shown is a partial restriction enzyme map of the murine β3-AR gene, the targeted β3-AR gene, and the two transgenes employed in the present study. Note that the targeted allele lacks 306 bp of β3-AR coding sequence, which is used in Northern blotting experiments to monitor β3-AR expression from the endogenous gene and the two transgenes. Large boxes refer to exons, the locations of which have been described previously (19, 24). The translated segments are shown in black. ATG and TAA represent β3-AR start and stop codons, respectively; AATAAA represents the β3-AR transcription termination and polyadenylation signal. The long, narrow, lightly shaded boxes represent the aP2 and UCP promoter/enhancer sequences. B, BamHI; Bg, BglII; E, EcoRI; H, HindIII; N, NheI; P, PstI, S, SalI; X, XhoI.

β3-AR mRNA Expression in Control, Knockout, WAT+BAT, and BAT-Transgenic Mice—Northern blotting was used to determine the expression level of β3-AR mRNA. The probe used for these studies corresponded to the 306-bp β3-AR coding sequence segment, which was deleted during the creation of the knockout allele (Fig. 1). Consequently, no signal was detected in RNA samples isolated from homozygous β3-AR knockout mice (Fig. 2). As expected, WAT+BAT-lines expressed β3-AR mRNA in white and brown adipose tissue while BAT-lines expressed β3-AR mRNA in brown adipose tissue only. With respect to white adipose tissue, line WAT+BAT-1 expressed at a level that was 44% above control mice while lines WAT+BAT-2 and WAT+BAT-3 expressed at levels that were 14 and 47% below control mice, respectively. With respect to brown adipose tissue, lines WAT+BAT-2 and WAT+BAT-3 expressed at levels that were approximately 2.5-fold higher than control mice, lines WAT+BAT-1 and BAT-1 expressed at levels that were just slightly above controls, while line BAT-2 expressed at a level that was slightly below control mice.

Northern blot analysis of total RNA isolated from brain, colon, kidney, liver, and skeletal muscle demonstrated that β3-AR mRNA expression, with the possible exception noted below, was restricted to adipose tissue (Fig. 3). In the case of BAT-lines, no expression was detected outside of brown adipose tissue (no signal in white adipose tissue, brain, colon, kidney, liver, and skeletal muscle). In the case of WAT+BAT-lines, however, low-level signals were occasionally detected in skeletal muscle but not in brain, colon, kidney, or liver. It is difficult to establish whether this apparent expression in skeletal muscle was due to ectopic expression in myocytes or represents

Fig. 2. Expression levels of β3-AR mRNA in brown and white adipose tissue. Total RNA was isolated from white and brown adipose tissue and was analyzed using Northern blotting techniques. The blots were probed using a 306-bp segment of β3-AR coding sequence deleted during the construction of the targeted allele. To quantitate β3-AR mRNA expression, radioactive signals from all Northern blots were submitted to PhosphorImager analysis (Molecular Dynamics, Image Quant software). Lanes that were not equally loaded as assessed by ethidium bromide fluorescence of 18 S and 28 S ribosomal bands were not analyzed. Expression in all groups was normalized to control mice. Results are expressed as mean ± S.E. The N for each group is as follows. Left panel (white fat): control (CONT), n = 12; knockout (KO), n = 8; WAT+BAT-1, n = 12; WAT+BAT-2, n = 12; WAT+BAT-3, n = 12; BAT-1, n = 6; and BAT-2, n = 6; right panel (brown fat): control, n = 12; knockout, n = 8; WAT+BAT-1, n = 3; WAT+BAT-2, n = 3; WAT+BAT-3, n = 3; BAT-1, n = 9; and BAT-2, n = 9.
expression in adipocytes that are resident within or surrounding this tissue. To address this issue, we carefully dissected away visible fat from skeletal muscle obtained from line WAT\(^{-}\). Northern blot analysis of these RNA samples failed to detect any specific \(\beta_3\)-AR mRNA signal, indicating that the weak signal observed in muscle samples from this line were likely due to the presence of adipocytes.

**Effect of CL on Oxygen Consumption**—Acute treatment with CL increased oxygen consumption in control mice by 89% (Fig. 4). As was previously observed (11), this response was completely absent in \(\beta_3\)-AR knockout mice (increase of 0%). In WAT+BAT-lines, the effect of CL on oxygen consumption was restored, with lines WAT+BAT-1 (increase of 120%) and WAT+BAT-2 (increase of 103%) having responses that were equal to or greater than control mice and line WAT+BAT-3 (increase of 60%) having a response that was somewhat less than control mice. CL also increased oxygen consumption in BAT-1 (increase of 24%) and BAT-2 (increase of 17%) transgenic mice; however, the \(O_2\) consumption response in these mice was notably less than that observed in control mice and in all three lines of WAT+BAT-transgenic mice.

**Effect of CL on Adenylate Cyclase Activity in Membranes**

**Fig. 3.** Expression of \(\beta_3\)-AR mRNA in brain (A), colon (B), kidney (C), liver (D), and skeletal muscle (E) and (F). Total RNA was isolated from tissues shown above and was analyzed using Northern blotting techniques and the probe described in Fig. 2. F, skeletal muscle dissection = RNA isolated from skeletal muscle following careful removal of surrounding white adipose tissue.
Control (KO), n = 13; BAT-1, n = 6; BAT+BAT-1, n = 4; BAT+BAT-3, n = 11; BAT-1, n = 7; and BAT-2, n = 6.

Isolated from Brown Adipose Tissue—Previously, it was assumed that the O2 consumption response to CL was mediated exclusively by β3-ARs in brown adipocytes. Therefore, the markedly reduced O2 consumption response of BAT-1 and BAT-2 mice was somewhat unexpected, especially since Northern blot expression data (Fig. 2) demonstrated that BAT-1 and BAT-2 mice have normal amounts of β3-AR mRNA in brown fat. However, it is formally possible that the mRNA transcribed from the UCP-β3-AR transgene bears an unknown mutation created during transgene construction or that the transgene-derived RNA transcript is translated at a lower efficiency due to differences in 5’-untranslated sequence. To rule out these important possibilities, we assayed for functional β3-ARs by assessing CL-mediated stimulation of adenylate cyclase activity in membranes isolated from interscapular brown adipose tissue of control, knockout, BAT-1, and BAT-2 mice. As previously reported (11), adenylate cyclase activity was not stimulated in membranes derived from knockout mice (Fig. 5). In contrast, adenylate cyclase activity was stimulated normally in membranes derived from BAT-1 and BAT-2 mice, demonstrating that BAT-mice express normal amounts of functional β3-ARs in brown adipose tissue and that the reduced O2 consumption response in these mice must be due to the absence of β3-ARs in white adipose tissue.

Effect of CL on Insulin, Glucose, and FFAs Levels—Previous studies have demonstrated that acute treatment of rodents with β3-AR selective agonists increases serum insulin levels by 10–100-fold (8, 11). To establish the time course for this effect, as well as for the effects on FFA and glucose levels, an initial experiment was performed on control mice (Fig. 6). This study demonstrated that FFA levels increased by 4.4-fold, with a near maximal increase being observed as early as 5 min. Insulin increased by 50-fold with a near maximal increase being observed at the 10-min point. A substantially smaller increase in insulin was observed at the 5-min point. Glucose concentrations decreased by 58%, with a near maximal decrease being observed at the 10-min point. At the 60-min point, insulin levels fell slightly but were still markedly elevated (increased by 24-fold) despite the presence of hypoglycemia during the preceding 50 min. The large increase in insulin levels and the persistent elevation despite hypoglycemia indicate that the stimulus for insulin secretion was potent. In all further experiments, FFAs, insulin and glucose were assessed at the 15-min point.

As previously reported (11), the ability of CL to increase FFAs and insulin and to reduce glucose was absent in β3-AR knockout mice (Fig. 7). These responses were completely restored in BAT+BAT-1 mice (FFAs increased by 3.6-fold, insulin increased by 91-fold, and glucose decreased by 55%), were only partially restored in WAT+BAT-2 mice (FFAs increased by 2.7-fold, insulin increased by 13-fold, and glucose decreased by 49%), and were minimally restored in WAT+BAT-3 mice (FFAs increased by 1.6-fold, insulin increased by 3.7-fold, and glucose decreased by 13%). Of note, as previously shown in Fig. 2, left panel, β3-AR mRNA expression in white adipose tissue was reduced in WAT+BAT-2 and WAT+BAT-3 mice, with the lowest level of expression being observed in line 3. Thus, it seems likely that decreased β3-AR gene expression in white adipose tissue accounts for the reduced responsiveness of these two lines. In BAT transgenic mice (BAT-1 and BAT-2), no effect of CL was observed on FFAs, insulin, and glucose, supporting the idea that β3-ARs in white adipose tissue are required for these responses.

Assessment of β3-AR and Actin mRNA Levels in Isolated Pancreatic Islets—Total RNA was extracted from control mouse pancreatic islets and was then assessed for β3-AR and actin mRNA expression using RT-PCR (data not shown). Total RNA from white adipose tissue was used as a positive control. β3-AR mRNA was not detected in RNA samples isolated from pancreatic islets but was detected in RNA samples derived from white fat. In contrast, actin mRNA was detected in both islet and white adipose tissue RNA samples. Thus, mouse pancreatic islets appear not to express detectable levels of β3-AR mRNA.

Effect of CL on Insulin Secretion from Isolated Pancreatic Islets—To rule out any possibility that CL stimulated insulin secretion via a direct effect on pancreatic β-cells, islets were isolated from control, knockout, and BAT+BAT mice and then treated with CL in the presence of submaximal stimulatory concentrations of glucose (11 mM). The secretory response at 11 mM glucose is one third to half of that obtained upon addition of arginine or an increase in glucose to 20 mM (21), demonstrating the sensitivity of this system for further stimulation of insulin secretion. As shown in Fig. 8, CL had no effect on insulin secretion in any group. This is in agreement with a previous study of isolated islets (8). Thus, the stimulatory effect of CL on insulin secretion is not mediated via stimulation of β3-ARs within islets.

Effect of CL on Food Intake—Acute treatment of control mice
with CL reduced food intake by 35–45% (Fig. 9, right and left panels). As was previously observed (11), this effect was completely absent in $\beta_3$-AR knockout mice. Significantly, the CL-mediated inhibition of food intake was observed in all WAT $\rightarrow$ BAT-transgenic mice (WAT $\rightarrow$ BAT-1, 89% inhibition; WAT $\rightarrow$ BAT-2, 94% inhibition; WAT $\rightarrow$ BAT-3, 63% inhibition). Of note, the degree of inhibition observed in each WAT $\rightarrow$ BAT transgenic line was greater than that observed in control mice. In contrast to WAT $\rightarrow$ BAT-transgenic mice, food intake was not inhibited in BAT-transgenic mice (BAT-1 or BAT-2 mice), consistent with the idea that $\beta_3$-ARs in white adipose tissue are required for the effect on food intake.

**DISCUSSION**

$\beta_3$-AR mRNA is expressed predominately in white and brown adipose tissue (4–7), and $\beta_3$-selective agonists are potential anti-obesity drugs (1–3). Chronic treatment of obese rodents with $\beta_3$-selective agonists decreases fat stores and improves obesity-linked insulin resistance. Acute treatment of rodents causes a number of diverse metabolic effects including an increase in oxygen consumption and insulin levels, and a decrease in food intake. The role of $\beta_3$-ARs, and $\beta_3$-ARs in white versus brown adipocytes in particular, in mediating each of these responses has been unclear. This is especially true given (a) the diverse nature of these responses, (b) the lack of clear explanations as to how stimulated adipocyte $\beta_3$-ARs might control insulin secretion and food intake, and (c) the recent observations suggesting the presence of $\beta_3$-ARs in non-adipocyte sites (5, 12–15). Recently, we generated gene knockout mice that lack functional $\beta_3$-ARs (11). These animals are completely resistant to CL-mediated effects on oxygen consumption, insulin secretion, and food intake demonstrating that each of these responses are mediated exclusively by $\beta_3$-ARs (11). To determine the role of white and brown adipocyte $\beta_3$-ARs in mediating these responses, we have combined gene knockout and transgenic techniques to create mice in which functional $\beta_3$-ARs are expressed selectively in white and brown adipocytes (WAT $\rightarrow$ BAT-mice) or in brown adipocytes only (BAT-mice). This was accomplished by injecting tissue-specific $\beta_3$-AR transgenic constructs (aP2-$\beta_3$-AR for WAT $\rightarrow$ BAT-mice and UCP-$\beta_3$-AR for BAT-mice) into mouse zygotes homozygous for the $\beta_3$-AR knockout allele (11). We then treated control, knockout, WAT $\rightarrow$ BAT, and BAT-mice with CL (1), the $\beta_3$-selective agonist, and determined effects on oxygen consumption, insulin secretion, and food intake.

The significant findings of this study are that transgenic re-expression of $\beta_3$-ARs in white and brown adipose tissue (WAT $\rightarrow$ BAT-mice) completely rescued CL-mediated effects on oxygen consumption, insulin levels, and food intake. Thus, each of these responses are mediated exclusively by $\beta_3$-ARs in white and/or brown adipocytes; receptors in non-adipocyte locations...
Role of β3-ARs on White and Brown Adipocytes

Aim: To investigate the role of β3-ARs on insulin secretion and energy expenditure in adipocytes. 

Methodology: In vitro studies were conducted using isolated white and brown adipocytes. The effects of CL on insulin secretion from pancreatic islets were also assessed. 

Results: CL increased insulin secretion from pancreatic islets, with the effect being more pronounced in brown adipocytes compared to white adipocytes. 

Conclusion: β3-ARs play a significant role in insulin secretion and energy expenditure, with brown adipocytes being more responsive than white adipocytes. 

Future studies will be needed to define the role of UCP2 in energy expenditure and the potential for direct effects of CL on pancreatic β-cells.
Role of β3-ARs on White and Brown Adipocytes

it is possible that CL-induced increases in FFAs (3–4-fold) mediate the effect on insulin release. Previous studies have demonstrated that FFAs are secretagogues for insulin secretion (29, 30), and the observed time course in the present study, i.e., increased FFA levels preceding increased insulin concentrations, is consistent with this possibility. However, the magnitude of the observed effect on insulin levels (50–100-fold increase), and its persistence despite the presence of hypoglycemia, seems to implicate additional factors as well. This is especially true since FFAs do not stimulate insulin secretion at low glucose levels, and starvation-induced increases in FFA concentrations do not lead to increased insulin levels. Thus, it is plausible that some novel factor emanating from white adipocytes, possibly some lipid product other than FFAs, directly or indirectly stimulates insulin secretion. Given the extreme potency of this stimulus, it will be important to identify the mechanism by which this response occurs.

Similarly, the mechanism by which CL treatment acutely decreases food intake is unknown. From the present study, it is clear that this response is mediated by β3-ARs in adipose tissue and requires the presence of β3-ARs on white adipocytes. The nature of this signal is also unknown. Leptin is a fat-derived protein that regulates appetite (31–34); however, it cannot account for this effect since leptin levels decrease substantially following β3-agonist treatment (35), and this would be predicted to have the opposite effect on food intake. Insulin is another factor that has been shown to suppress food intake (36, 37), and as discussed above, insulin levels rise substantially following treatment with CL. However, insulin may not be mediating the suppression of food intake since in WAT the transgenic line 3, a minimal effect was observed on food intake. Heat has long been known to have effects on regulating food intake. Heat production is predicted to be markedly increased in all tissues that had decreased food intake (control mice and WAT–BAT mice, but not BAT mice). If heat is the signal mediating the suppression of food intake, then it is predicted that it requires the presence of β3-ARs in white as well as brown adipocytes. For this and other reasons, it would be of interest to create mice that express β3-ARs in white adipocytes only. Finally, other possible candidates for mediating the effects of CL on food intake are lipid fuels (FFAs, or ketone bodies) or possibly another fat-derived protein capable of regulating food intake. Future work will focus on identifying the mechanism responsible for this effect.

In summary, acute treatment with β3-selective agonists produces a number of diverse metabolic effects, including stimulation of O2 consumption, insulin secretion, and inhibition of food intake. In the present study, we have used a transgenic and gene knockout approach to genetically identify the receptor and tissues responsible for mediating each of these important responses. From this and an earlier study (11), we can conclude that each of these effects are mediated exclusively by β3-ARs on adipocytes. In addition, β3-ARs in white adipocytes must be present to obtain any effect on insulin secretion and food intake and for a full effect on energy expenditure. Determining the mechanisms by which stimulation of adipocytes produces each of these responses will be the focus of future studies.

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