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Norepinephrine Induces Lipolysis in $\beta_1/\beta_2/\beta_3$-Adrenoceptor Knockout Mice

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ABSTRACT

Catecholamines are major stimulants of adipose tissue metabolism. Norepinephrine and epinephrine act through three subtypes of β-adrenoceptors (β-AR) expressed in the adipocytes. The aim of this work was to study the mechanisms of lipid mobilization in $\beta_1/\beta_2/\beta_3$-AR triple-knockout (β-less) mice. Glycerol and nonesterified fatty acids released from isolated adipocytes were measured as an index of lipolytic activity. There was no difference between the two genotypes for basal lipolysis and lipolytic response to corticotropin or to agents acting at the adenylyl cyclase and protein kinase A levels. The lipolytic response to norepinephrine and β-AR agonists was blunted in β-less mice. However, a residual low-affinity lipolytic effect was observed in the presence of catecholamines and β_3-AR agonists but not of β_1- or β_2-AR agonists. cAMP levels were increased by a β-AR agonist in white and brown adipocytes of β-less mice. The residual lipolytic effect was blocked by β-AR antagonists. It was mediated neither by α_1- or α_2-AR nor by dopaminergic, serotonergic, and histaminergic receptors. Bioinformatic analyses do not provide evidence for a fourth β-AR. We conclude that the residual lipolytic effect observed in β-less mice can be attributed to an unknown Gs-protein–coupled receptor with low affinity for catecholamines.

Factors that control the storage and mobilization of triglycerides in adipose tissue are important regulators of fat accumulation. Adipose tissue lipolysis [i.e., the catabolic process leading to the breakdown of triglycerides into nonesterified fatty acids (NEFA) and glycerol] is under the control of the sympathetic nervous system. Norepinephrine, the neurotransmitter, and epinephrine, the hormone secreted by adrenal glands, act via three subtypes of β-AR expressed on the fat cells. These three subtypes are coupled positively to adenylyl cyclase via Gs proteins and mediate, through an elevation of intracellular cAMP levels and activation of protein kinase A, the phosphorylation and activation of hormone-sensitive lipase, the rate-limiting enzyme of adrenoceptor-stimulated lipolysis (Langin et al., 2000). Redundancy between the three receptors was best exemplified by the phenotypes of simple β-AR gene knockout models in mice. Indeed, β_1-AR, β_2-AR, or β_3-AR knockout mice did not display an overt obesity and major lipid metabolism disturbances (Susulic et al., 1995; Rohrer et al., 1996; Revelli et al., 1997; Chruscinski et al., 1999). However, $\beta_1/\beta_2/\beta_3$-adrenoceptor knockout (β-less) mice became mildly obese on a chow diet and more overtly obese on a high-fat diet (Bachman et al., 2002; Jimenez et al., 2002). This phenotype could partly be explained by a defect in the sympathetic nervous system-dependent activation of diet-induced thermogenesis in brown adipose tissue (Bachman et al., 2002). It is surprising that the lipolytic response to fasting was not altered in these mice (Jimenez et al., 2002).

To study the mechanism of fat mobilization from white adipose tissue in β-less mice, we investigated the action of adrenergic and nonadrenergic activators of lipolysis on isolated adipocytes in vitro. Here, we show that norepinephrine is able to stimulate lipolysis in the absence of β-ARs and

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ABBREVIATIONS: NEFA, nonesterified fatty acids; β-AR, β-adrenoceptor; β-less, $\beta_1/\beta_2/\beta_3$-adrenoceptor triple knockout; dbcAMP, dibutyryl cAMP; WT, wild type; KRBA, Krebs-Ringer bicarbonate buffer containing albumin, glucose, and HEPES; CL316243, (*R*,*R*)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate; (±)-RX 821002, 2-methoxy-idazoxan; CGP12177, 4-[3-*t*-butylamino-2-hydroxypropoxy] benzimidazol-2–1; CGP20712A, 2-hydroxy-5-(2-(hydroxy-3-(4-((1-methyl-4-trifluoromethyl)-1*H*-imidazol-2-yl)phenoxy)propyl)aminoethoxy)benzamide; RX821002, 2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole.

present a characterization of this residual lipolytic effect of catecholamines in white fat cells.

Materials and Methods

Generation of β-Less Mice. Animals were treated in accordance with the Centre Médical Universitaire (Genève, Switzerland) and Institut Louis Bugnard (Toulouse, France) institutional guidelines. β-Less ($\beta_1^{-/-}\beta_2^{-/-}\beta_3^{-/-}$) and wild-type (WT) ($\beta_1^{+/+}\beta_2^{+/+}\beta_3^{+/+}$) strains were obtained by intercrossing our β_3-AR knockout mice (Revelli et al., 1997) and β_1/β_2-AR knockout mice (Rohrer et al., 1999), kindly provided by Dr B. K. Kobilka (Howard Hughes Medical Institute, Stanford, CA). $\beta_1^{+/-}\beta_2^{+/-}\beta_3^{+/-}$ offspring were crossed to generate $\beta_1^{+/+}\beta_2^{+/+}\beta_3^{+/+}$ and $\beta_1^{-/-}\beta_2^{-/-}\beta_3^{-/-}$ mice, from which were established WT and β-less colonies on the same, mixed genetic background (129 Sv/ev, 129 Sv/J, FVB/N, C57BL/6J, and DBA/2). Genotypes were determined by Southern blot. Studies were performed on male mice.

Drugs. Bovine serum albumin (fraction V), clenbuterol, collagenase, dobutamine, dopamine, forskolin, glucagon, histamine, (−)-isoproterenol, (−)-norepinephrine, (±)-*p*-octopamine, (−)-propranolol, *R*-(−)-phenylisopropyladenosine, serotonin, tyramine, and all antioxidants were from Sigma-Aldrich (St. Quentin Fallavier, France). Corticotropin was from Novartis Pharma SA (Basel, Switzerland). Adenosine deaminase was from Roche (Meylan, France). Dibutyryl-cAMP (dbcAMP) was from Roche. (−)-Bupranolol was from Schwarz Pharma (Monheim, Germany). CL316243 was from the Medical Research Division, Lederle Laboratories, American Cyanamid Company (Pearl River, NY). Prazosin was from Pfizer, Inc. (La Jolla, CA). (±)-RX 821002 was from Fabre (Castres, France). Phentolamine was from Aventis (Rueil-Malmaison, France). [γ-^{32}P]ATP was from PerkinElmer Life and Analytical Sciences (Boston, MA). Determination of cAMP was performed using enzyme immunoassay kits from Cayman Chemical Company/SPI-BIO (Massy, France).

Lipolysis Assay. Animals were killed after an overnight fast according to Institut National de la Santé et de la Recherche Médicale animal care ethical guidelines. Isolated adipocytes were obtained by collagenase digestion (1 mg/ml) of visceral fat pads in Krebs-Ringer bicarbonate buffer containing albumin (3.5 g/100 ml), glucose (108 mg/100 ml), and HEPES (238 mg/100 ml) at pH 7.4 (KRBA) and under vigorous shaking at 37°C (Lucas et al., 2003; Rodbell, 1964). Then, the fat cells were filtered through a nylon screen and washed three times with KRBA buffer to eliminate collagenase. A similar protocol was used to isolate brown adipocytes from the interscapular fat pad with collagenase used at 10 mg/ml and a modified KRBA buffer containing albumin (1 g/100 ml), glucose (49 mg/100 ml), and HEPES (477 mg/100 ml) (Atgie et al., 1997). Isolated adipocytes were brought to a 1/20 dilution in KRBA buffer with adenosine deaminase (1 U/ml) and *R*(−)-phenylisopropyladenosine (100 nM) for lipolysis assays and incubated with the pharmacological agents in a final volume of 100 μl for 90 min at 37°C under gentle shaking. At the end of the incubation, 30- and 5-μl aliquots of the infranatant were taken for glycerol and NEFA determination using a radiometric assay (Bradley and Kaslow, 1989) and the NEFA C kit (Wako, Oxoid SA, France), respectively. Total lipid was determined gravimetrically after solvent extraction (Lucas et al., 2003).

Determination of cAMP Concentrations. Fat cells were preincubated in 500 μl of KRBA at 37°C for 90 min in the presence of isoproterenol. The addition of a solution of chloroform, methanol, and 1 M HCl (2:1:0.1 v/v) containing 0.5 mM 3-isobutyl-1-methylxanthine (a nonselective phosphodiesterase inhibitor) stopped the reaction. After centrifugation (5000 rpm, 5 min), the aqueous phase of each sample was collected, freeze-dried, and redissolved in the buffer of the enzyme immunoassay kit according to the manufacturer's instructions for cAMP determinations.

Data Analysis and Statistics. Values are given as means ± S.E.M. The Mann-Whitney nonparametric test was used for comparisons between genotypes. The Wilcoxon nonparametric test was used for paired comparisons in each genotype. A p value <0.05 was the threshold of significance. EC_{50} values were obtained by computer fitting of concentration-response curves to various β-AR agonists and corticotropin.

Bioinformatic Analysis. The complete sets of human and mouse proteins were recovered from the European Bioinformatics Institute website (http://www.ebi.ac.uk). We used the PROSITE profile PS50262 to identify all human and mouse G-protein–coupled receptor class A proteins (http://www.expasy.org/cgi-bin/prosite-search-ac?PDOC00210) and to generate the corresponding multiple sequence alignment. We then used the multiple sequence alignments to build the corresponding phylogenetic trees with the ClustalW program (Neighbor Joining method). We extracted the 50 proteins closest to the β-AR and generated a second tree. The mouse subset was also blasted against the human one to check consistencies between the two samples.

Results

Body and Tissue Weights in β-Less and WT Animals. Body and tissue weights are shown in Table 1. We observed no differences in body weight between WT and β-less mice. However, the different white fat pads were heavier in β-less than in WT male mice. Hypertrophy of brown adipose tissue was also observed.

Basal and Maximal Lipolysis. Lipolysis experiments were exclusively performed using visceral fat pads (i.e., pooled epididymal and perirenal white adipose tissues). Glycerol and NEFA releases were used as indexes of lipolytic activity. There was no difference between genotypes (WT, $n = 20$; β-less, $n = 20$) in basal values for glycerol and NEFA levels (0.66 ± 0.02 versus 0.76 ± 0.02 and 0.76 ± 0.11 versus 1.08 ± 0.15 μmol/90 min/100 mg of lipid, respectively). WT and β-less mice showed similar maximal lipolysis induced by various agents acting at different steps of the lipolytic cascade such as forskolin, an activator of adenylyl cyclase; dbcAMP, a stable analog of cAMP which activates protein kinase A; and corticotropin, which binds to a Gs-protein–coupled receptor (Fig. 1).

As reported previously (Carpéné et al., 1999), norepineph-

TABLE 1
Body and tissue weights in WT and β-less mice
Weights were measured on 14 WT and 15 β-less animals.

Status	Body	Adipose Tissue					Liver
		Subcutaneous	Epididymal	Perirenal	Total White	Brown	
				g			
WT	37.55 ± 1.06	0.94 ± 0.11	1.19 ± 0.13	0.34 ± 0.03	2.37 ± 0.28	0.119 ± 0.014	1.31 ± 0.07
β-less	34.36 ± 1.37	1.49 ± 0.12**	1.5 ± 0.12*	0.46 ± 0.04*	3.19 ± 0.3*	0.528 ± 0.089***	1.13 ± 0.06

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

rine, the nonselective β-AR agonist isoproterenol, and selective β-AR agonists (dobutamine for β_1-AR, clenbuterol for β_2-AR, and CL316243 and octopamine for β_3-AR) strongly stimulated lipolysis in WT mice (Fig. 2). Dopamine showed a weak lipolytic effect in WT mice. No lipolysis was elicited by the β_1- and β_2-AR agonists in β-less mice. A residual β-AR lipolytic effect was observed in β-less mice using norepinephrine, isoproterenol, CL316243, and octopamine reaching, respectively, 5.1 ± 0.7-, 4.9 ± 1.1-, 2.4 ± 0.3-, and 1.8 ± 0.4-fold over the basal values for glycerol release. Similar results were obtained for NEFA (data not shown). Various antioxidants did not modify the response to norepinephrine in β-less mice, ruling out the possibility that the lipolytic effect might be caused by the oxidation of catecholamines (data not shown).

Determination of cAMP Levels in White and Brown Adipocytes of β-Less Mice. It was important to determine whether the residual lipolytic effect observed in β-less mice was associated with a cAMP-dependent signaling pathway. As shown in Fig. 3A, isoproterenol led to an increase of cAMP levels in β-less white adipocytes. Figure 3B shows that the residual stimulatory effect of isoproterenol on cAMP production is also present in brown adipocytes.

Characterization of the Residual β-AR Lipolytic Effects. We further investigated the residual lipolytic effect of norepinephrine and β-AR agonists. White adipocytes from WT and β-less mice were incubated with increasing doses of various agents, and glycerol release was measured. Norepinephrine and isoproterenol EC_{50} values were 14-fold higher in β-less than in WT mice, whereas the CL316243 value was only 3-fold higher in β-less than in WT mice (Table 2). Similar results were obtained for NEFA release (data not shown). The rank order of potency was therefore decrescendo: CL316243 > isoproterenol > norepinephrine in WT and β-less mice.

Next, we investigated the inhibition of norepinephrine-induced residual lipolytic effect by various adrenergic antagonists. The lipolytic stimulation induced by 10 μM norepi-

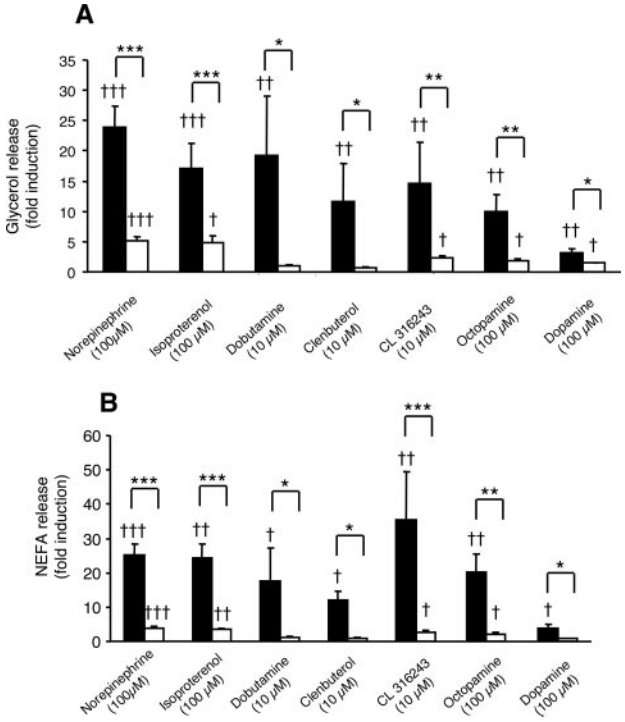

Fig. 2. Maximal lipolysis in adipocytes from WT (■) and β-less (□) mice with various β-AR agonists. Results are expressed as fold induction over basal levels. Values are means ± S.E.M. of 9 to 20 animals. Mann-Whitney nonparametric statistical test was used to compare β-less and WT animals; ⋆, $p < 0.05$; ⋆⋆, $p < 0.01$; ⋆⋆⋆, $p < 0.001$. Wilcoxon nonparametric statistical test was used to compare the effect of each drug with basal lipolysis in β-less or WT mice; †, $p < 0.05$; ††, $p < 0.01$; †††, $p < 0.001$. A, glycerol release. B, NEFA release.

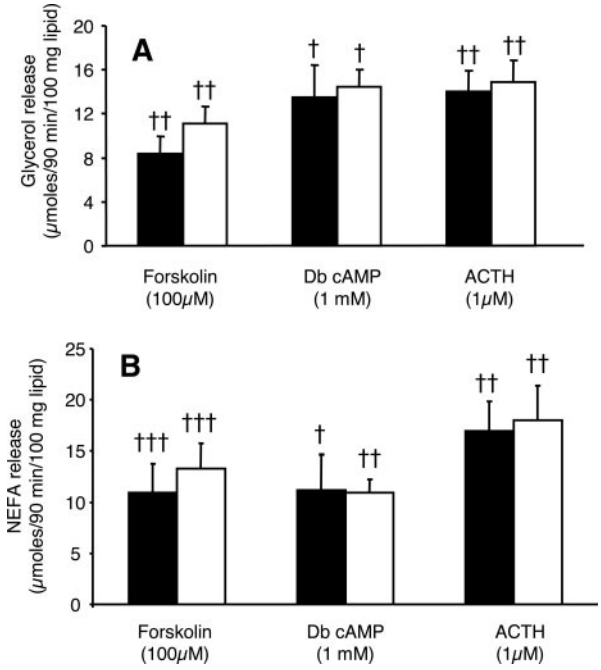

Fig. 1. Maximal lipolysis in adipocytes from WT (■) and β-less (□) mice with various pharmacological agents acting at different steps of the lipolytic cascade. Values are means ± S.E.M. of 7 to 15 animals. Wilcoxon nonparametric statistical test was used to compare the effect of each drug with basal lipolysis in β-less or WT mice; †, $p < 0.05$, ††, $p < 0.01$; †††, $p < 0.001$. A, glycerol release. B, NEFA release. ACTH, corticotropin.

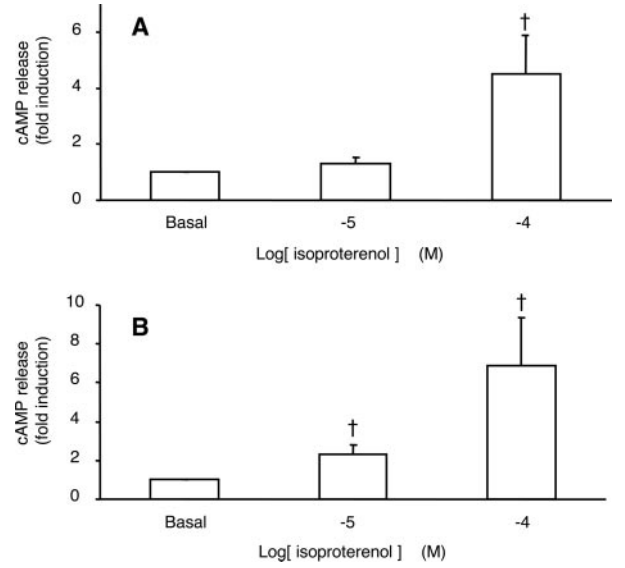

Fig. 3. cAMP levels in isolated white (A) and brown (B) adipocytes from β-less mice. Results are expressed as fold induction over basal levels. Values are means ± S.E.M. of four and five animals for white and brown adipocytes, respectively. Wilcoxon nonparametric statistical test was used to compare the effect of isoproterenol with basal lipolysis. †, $p < 0.05$.

nephrine was not antagonized in WT and β-less mice by α_1- (prazosin) and α_2- (RX821002) and mixed α_1/α_2- (phentolamine) AR antagonists (Fig. 4). No inhibition was observed using 100 μM norepinephrine (data not shown). The β_1/β_2-AR antagonist propranolol and the nonselective β-AR antagonist bupranolol inhibited norepinephrine-induced NEFA release in the two genotypes, although with a lower potency in the case of bupranolol in β-less mouse adipocytes (Fig. 5). Similar results with α- and β-AR antagonists were obtained for glycerol release (data not shown).

Lipolysis Induced by Nonadrenergic Agents. There was no difference in corticotropin EC_{50} values between β-less and WT mice (Fig. 6 and Table 2). Various agents acting via G protein-coupled receptors (histamine, serotonin, tyramine, and glucagon) were tested on the isolated adipocytes of β-less and WT mice (Martinez-Conde et al., 1984; Raimondi et al., 1993; Tebar et al., 1996; Bairras et al., 2003). No stimulation of lipolysis over basal value was observed after 90 min (Fig. 7).

Sequence Comparison of Class A G-Protein–Coupled Receptors. A search was performed for proteins corresponding to class A G-protein–coupled receptors. Here, 925 human proteins and 1858 mouse proteins were identified. A phylogenetic tree was built. Figure 8 shows β-AR and related receptors in the mouse. The most proximal proteins of β-AR were receptors for serotonin, adenosine, histamine, trace amines, and dopamine. No novel proteins cluster to the β-AR. We thus found no evidence for a putative new β-AR. An analysis of human sequences led to similar conclusions (data not shown).

Discussion

Catecholamines are important lipolytic hormones acting in rodents through the three β-AR subtypes (β_1-, β_2-, and β_3-AR). By homologous recombination and further intercrosses, mice invalidated for the three known β-ARs were produced (Jimenez et al., 2002). White and brown fat pad of these β-less male animals were heavier, as shown previously (Bachman et al., 2002; Jimenez et al., 2002). Visceral fat pads were used in in vitro lypolytic experiments. There was no difference in basal lipolysis between β-less and WT mice. Maximal lipolysis induced by pharmacological drugs acting at a postreceptor level in the lipolytic pathway (i.e., forskolin, a direct activator of the adenylyl cyclase or dbcAMP, a stable analog of cAMP activating directly protein kinase A) were equally strong in both genotypes. Moreover, corticotropin lipolytic effect was as potent in β-less as in WT mice. As expected, β_1-and β_2-AR agonists produced no glycerol or NEFA release in β-less mice. We were surprised to find a 5-fold residual lipolytic effect induced by the natural catecholamine norepinephrine and by the nonselective β-AR agonist isoproterenol in β-less animals. There was also a weak (2-fold) lipolytic effect induced by the β_3-AR–selective agonist CL316243. Octopamine can also be considered as a β_3-AR agonist. It stimulates lipolysis in all species expressing this receptor subtype, and its effect is resistant to blockade by β_1- and β_2-AR antagonists (Galitzky et al., 1993). In β-less mice, there was a weak (2-fold) but significant octopamine residual effect. Dopamine, an endogenous catecholamine, has been shown to exert no (in rat, hamster, dog, and humans) or a

TABLE 2

Potency of various lipolytic agents in WT and β-less white adipocytes

Dose-response curves were built with concentrations ranging from 100 pM to 100 μM (norepinephrine and isoproterenol), from 100 pM to 10 μM (CL316243), and from 10 pM to 10 μM (corticotropin). Glycerol release was used as an index of lipolysis.

Drugs	Norepinephrine	Isoproterenol	CL316243	Corticotropin
		nM		
WT (n = 3–8)	1188 ± 398	462 ± 146	75 ± 45	29 ± 15
β-less (n = 3–8)	16326 ± 12479*	6246 ± 5572*	223 ± 37*	42 ± 10

* $p < 0.05$.

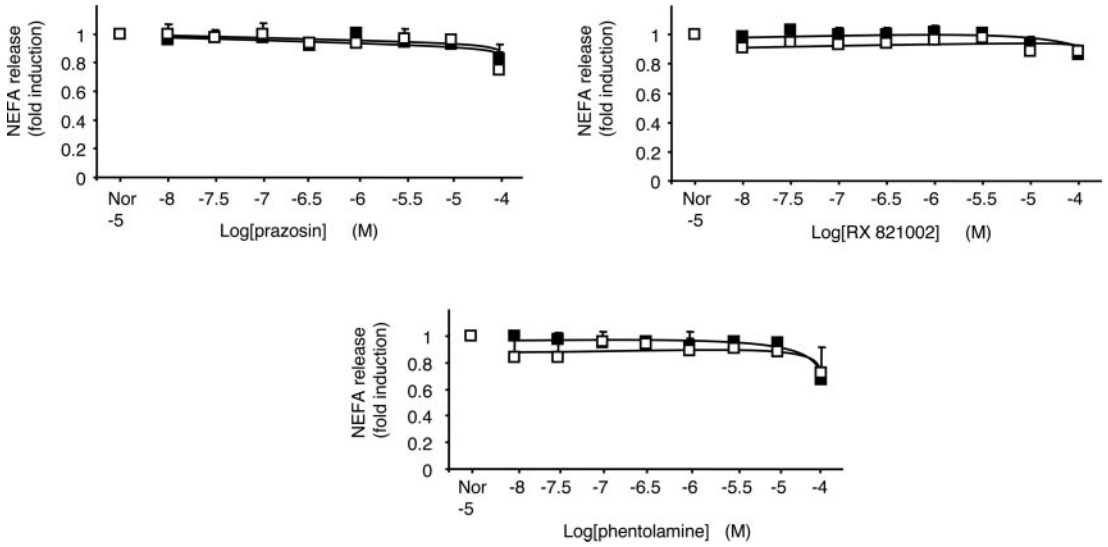

Fig. 4. Dose-response curves of α-AR antagonists on maximal NEFA release in adipocytes from WT (■) and β-less (□) mice. Results are expressed as fold induction of the norepinephrine-induced maximal lipolysis. Values are means ± S.E.M. of four animals.

small (in guinea pig) lipolytic action at a 1 mM concentration in white adipose tissue (Carpéné et al., 1999). In mice, there was a small effect in WT animals and a weaker but still significant effect in β-less mice. In β-less mice, EC_{50} values for norepinephrine, isoproterenol, and CL316243 are high, suggesting the existence of a low-affinity catecholamine receptor. As suggested for the β_3-AR, recruitment of the putative receptor at high concentrations of catecholamines suggests that this receptor is operative in situations of high sympathetic nervous system activation (Giacobino, 1995). Inhibition curves for propranolol, a β_1- and β_2-AR antagonist, were superimposable in β-less and WT mice. If a β-AR antagonist shows similar potency for conventional β-AR and the residual receptor, the concentration of the antagonist needed to block the agonist effect should be lower in β-less than in WT mice, because the EC_{50} values for agonists are higher in β-less than in WT mice. Hence, propranolol seems to be more efficient for WT than for β-less mice. On the other hand, bupranolol, a nonselective β-AR antagonist, has low antagonist potency toward the residual receptor. Different α-AR antagonists had no effect. The receptor with low affinity for catecholamines presents the pharmacological profile of an atypical β-AR.

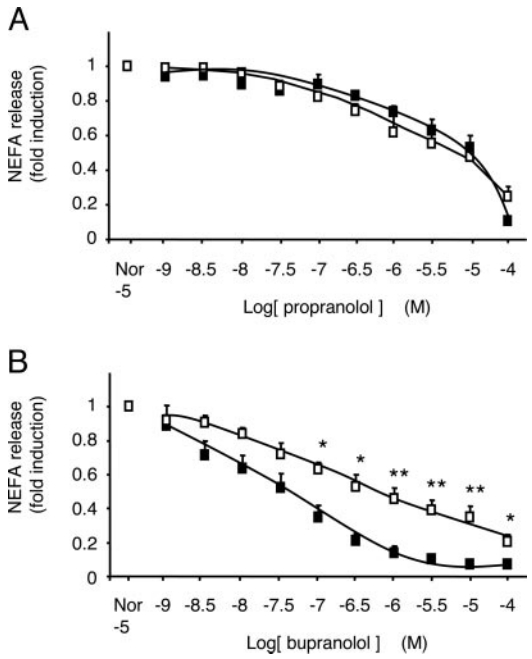

Fig. 5. Dose-response curves of β-AR antagonists on maximal NEFA release in adipocytes from WT (■) and β-less (□) mice. Results are expressed as fold induction of the norepinephrine-induced maximal lipolysis. Values are means ± S.E.M. of six animals. Mann-Whitney nonparametric statistical test was used to compare β-less and WT animals. ⋆, $p < 0.05$; ⋆, $p < 0.01$; ⋆⋆⋆, $p < 0.001$.

The existence of a "β_4-AR" has been suggested in white adipose tissue on the basis of the effect of CGP12177 (Galitzky et al., 1997, 1998). CGP12177 is a β_1- and β_2-AR antagonist with β_3-AR partial agonist activity (Langin et al., 1991). In rat and human fat cells, it has been shown that CGP12177 can activate lipolysis through the interaction with a β-AR that is pharmacologically distinct from the β_3-AR (Galitzky et al., 1997). In vivo pharmacological studies with β_3-AR knockout mice revealed that CGP12177 induces tachycardia and increases oxygen consumption (Ito et al., 1998; Kaumann et al., 1998; Cohen et al., 2000). These data were confirmed by the in vitro thermogenic response of the brown adipose tissue of β_3-AR knockout mice (Preitner et al., 1998). These different results suggested that CGP12177 acted through a novel β-AR. However, pharmacological analysis with Chinese hamster ovary cells expressing human and rat β_1-AR showed that CGP12177 activation of adenylyl cyclase is blocked by CGP20712A, a β_1-AR selective antagonist (Granneman, 2001). Furthermore, measurements of adenylyl cyclase activity in brown adipose tissue of β_1- and β_3-AR knockout mice indicated that CGP12177 effects were mediated by an atypical interaction with β_1-AR (Konkar et al., 2000; Granneman, 2001). This is also the case in the cardiovascular system. Using double β_1- and β_2-AR knockout mice, Kaumann et al. (2001) concluded that CGP12177 cardiostimulant effect could be mediated by a novel state of the β_1-AR. In β-less mice, we did not observe any lipolytic effect of CGP12177 (data not shown). The residual lipolytic effect elicited by β-AR agonists in β-less mice is antagonized by propranolol, whereas the putative "β_4-AR" is not sensitive to this antagonist. Finally, the effect observed in β-less mice is stimulated by β_3-AR agonists, a feature differing from that of the putative β_4-AR. The residual lipolytic activity is therefore clearly distinct from the β_4-AR lipolytic effect.

The nature of the new receptor remains elusive. The increase in intracellular levels of cAMP induced by isoproterenol in white adipocytes of β-less mice suggests that the residual receptor is coupled to adenylyl cyclase via Gs proteins. Because the β-less mice display marked changes in the phenotype of their brown adipose tissue (Bachman et al., 2002; Jimenez et al., 2002), it was interesting to test the existence or not of a residual receptor in this tissue. Our results showed that a residual receptor coupled to adenylyl cyclase via Gs proteins, similar to that found in the white adipocytes, is

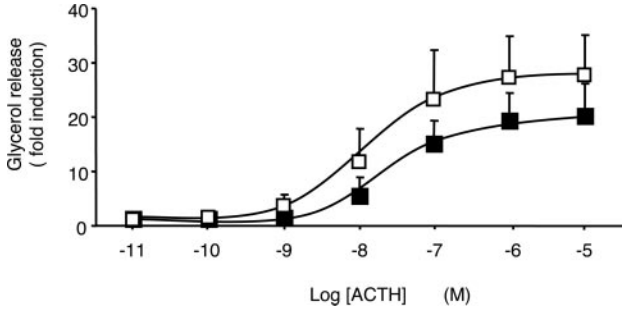

Fig. 6. Dose-response curve of corticotropin (ACTH) on glycerol release in adipocytes from WT (■) and β-less (□) mice. Results are expressed as fold induction over basal levels. Values are means ± S.E.M. of eight animals.

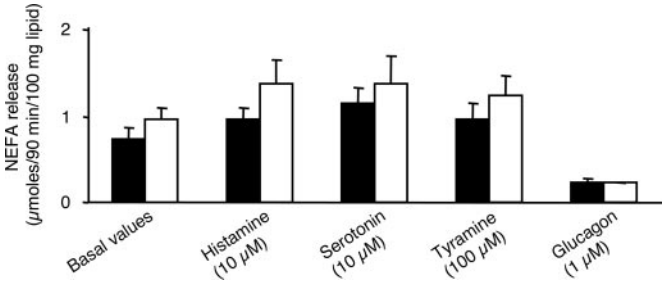

Fig. 7. Maximal lipolysis in adipocytes from WT (■) and β-less (□) mice with various pharmacological agents. NEFA concentrations in the medium were determined. Values are means S.E.M. of 5 to 15 animals.

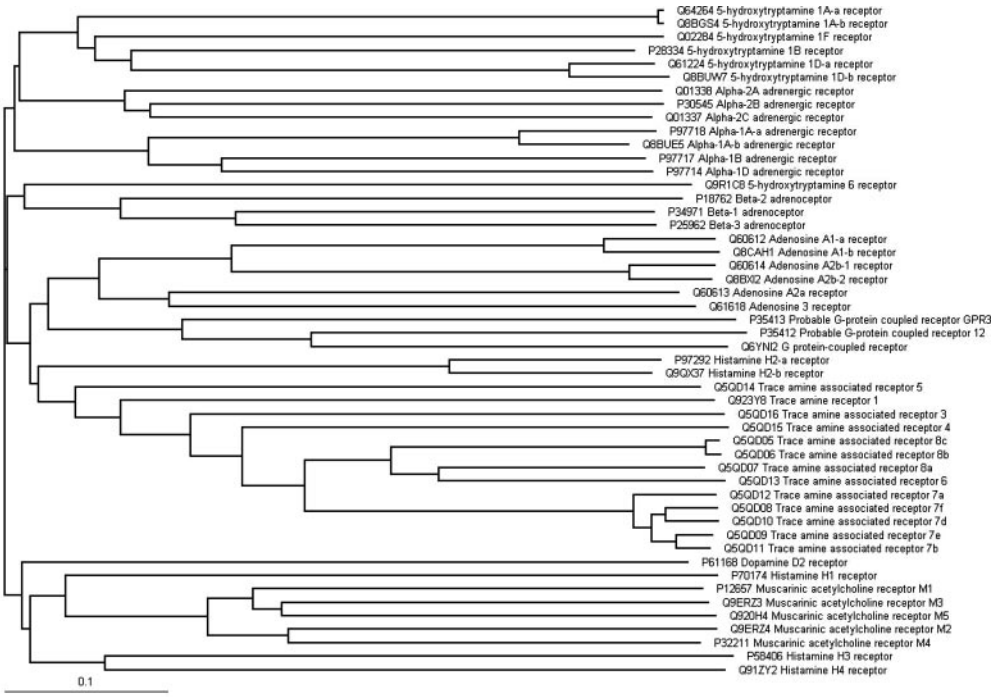

Fig. 8. Local phylogenetic tree for mouse adrenoceptors and related class A G-protein–coupled receptors. The first six alphanumerical characters correspond to the UniProt accession numbers, which is followed by the name of the protein. Scale, 0.1 correspond to 10% of divergence.

present in β-less brown adipocytes. The in silico study using the human and mouse G-protein–coupled receptor sequences provided no evidence for a new β-AR. Closely related Gs-coupled receptors comprised dopaminergic, serotonergic, and histaminergic receptors. Because there is only a weak lipolytic effect of dopamine and no stimulation with histamine and serotonin, these receptors are not likely to correspond to the receptor activated by high concentrations of β-AR agonists in β-less mouse adipose tissue. Adenosine receptors showed significant homology. The adenosine A1 receptors are expressed in human fat cells. They are, like α_2-adrenoceptors, coupled to Gi and are involved in antilipolysis (Lafontan and Berlan, 1995). Some degrees of homology were found with probable G protein-coupled receptors (i.e., trace amine receptors and orphan receptors belonging to the class A of G-protein–coupled receptors) (Borowsky et al., 2001). Tyramine, an activator of trace amine receptors 1 and 2, had no effect on lipolysis. At present, it is difficult to speculate on the possible involvement of one of these receptors.

There is an interesting similarity between our observation in β-less mice and what has been observed in human adipocytes. We showed previously that β_3-ARs do not contribute to norepinephrine-mediated lipolysis in human subcutaneous adipocytes (Tavernier et al., 1996; Galitzky et al., 1997). However, CL316243 displays an EC_{50} value similar to that found in β-less mice, suggesting the existence in human adipocytes, besides the β_1- and β_2-AR, of a low-affinity receptor. The nature and the physiological role of this novel receptor remain to be determined.

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