0026-895X/92/040634-04$03.00/0

MOLECULAR PHARMACOLOGY, 42:634-637

Functional Properties of the Rat and Human β_3-Adrenergic Receptors: Differential Agonist Activation of Recombinant Receptors in Chinese Hamster Ovary Cells

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Received June 19, 1992; Accepted July 20, 1992

SUMMARY

β_3-Adrenergic receptors (β_3AR) mediate lipolytic and thermogenic responses in rodent adipose tissues *in vitro*, and "atypical" βAR agonists that active these receptors have potent therapeutic effects in *in vivo* rodent models of adult-onset diabetes and obesity. However, experiments with rodent cells that natively express the β_3AR, as well as those with cells that express cloned rodent β_3AR, have suggested that the pharmacological properties of the rodent and human β_3AR differ. Given that rodent models of obesity and diabetes are used to develop human therapeutic agents, we sought to compare directly the ligand-binding and functional properties of the rat and human β_3AR in parallel studies using Chinese hamster ovary cells expressing the recombinant receptors. The endogenous catecholamines epinephrine (EPI) and norepinephrine (NE) were found to have low affinities (micromolar) for the β_3AR of both species. The rank orders of potency of various agonists in stimulating adenylyl cyclase were clearly different, i.e., for the human β_3AR, CGP12177 (CGP) > isoproterenol (ISO) ≥ BRL34377 (BRL) = Pindolol > NE > EPI; for the rat, CGP ≥ BRL > ISO ≥ NE > Pindolol > EPI. The intrinsic activities of various agonists were also different, with the following rank orders (compared with ISO): for the human β_3AR, NE > EPI > BRL = CGP > Pindolol; for the rat β_3AR, BRL > NE > EPI > CGP > Pindolol. Competition binding studies with ^{125}I-cyanopindolol and these agonists gave similar rank orders of potency. Thus, although the human and rat receptors exhibited similar properties with respect to catecholamine agonists, numerous differences in the potency and efficacy of synthetic noncatecholamine agonists were noted, indicating that the action of atypical agonists at rodent β_3AR may not be predictive of therapeutic potential in humans.

Rat adipose tissues contain "atypical" βAR, which have long been the focus of investigations of metabolic responses that are mediated by adrenergic agents (1, 2). More recently, these receptors have gained attention as potential therapeutic targets of specific agonists that might provide antidiabetic, thermoregulatory, or antiobesity properties (3, 4). The vast majority of studies that have characterized atypical receptors have used rodent adipose tissue (which express all three known βAR subtypes), and these rodent receptors have been considered the prototypical atypical receptors (5).

The cloned human β_3AR has been reported to possess several pharmacological features that are found in rodent atypical receptors (5). However, numerous differences between rodent atypical receptors and the cloned human β_3AR have been noted, leading some to question whether they were, in fact, homologous proteins (6, 7). Recently, the rat β_3AR has been cloned and expressed, and the results of pharmacological studies have raised further doubts regarding the pharmacological similarity of the human and rodent homologs (8, 9).

Given that agonists of the β_3AR are being developed as potential therapeutic agents for diabetes and obesity and these drugs are often first developed in rodent models of these diseases, it is important to determine whether the agonist sensitivities of the human and rat β_3AR are the same. The reported pharmacological differences between the cloned rat and human receptors have relied entirely upon the initial characterization of the human receptor (5), with the human receptor data being republished several times (9–11). The only other data that are available concerning the cloned human receptor compared it with a murine atypical βAR that is natively expressed (along with β_1AR and β_2AR) in the 3T3-F442A cell line (12). However, the data on the human receptor reported in this latter paper are at variance in several respects (see Discussion) with the original characterization of the human receptor by the same authors (5) and, in any event, they did not compare the cloned receptors from both species. Thus, the pharmacological rela-

This work was supported in part by National Institutes of Health Grant HL-45967.

ABBREVIATIONS: βAR, β-adrenergic receptors; ICYP, ^{125}I-cyanopindolol; K_i, equilibrium dissociation constant; K_{act}, activation constant; ISO, isoproterenol; EPI, epinephrine; NE, norepinephrine; CGP, CGP12177; BRL, BRL34377; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; CHO, Chinese hamster ovary.

tionship between the rodent and human receptors remains confusing, if not contradictory, in large part because no experiments have been reported that compared the cloned receptors of rodents and humans simultaneously under identical assay conditions. Therefore, we undertook the current study by expressing the rat and human β_3AR in Chinese hamster ovary cells (which do not naturally express any βAR), and we determined key pharmacological properties in parallel studies, thereby resolving this previously confusing area.

Materials and Methods

Receptor cloning and cell culture. The cloning of the rat β_3AR and the generation of cells that stably express this receptor have been reported previously (8). The human β_3AR was cloned as described (13) from a human leukocyte genomic library, using a probe based upon the published sequence (5). Expression of human β_3AR in CHO cells was accomplished by co-transfection with 30 μg of β_3AR DNA (in the expression vector pBC12BI) and 2.0 μg of pSV_2Neo (which provides G418 resistance) using calcium phosphate precipitation, as described (14) for expression of β_1- and β_2AR. Stable transformants were selected in 1 mg/ml G418. Clonal lines were screened with an ICYP binding assay similar to that previously described (14), using 1.0 mM ISO to define nonspecific binding. For the current study, CHO cells expressing the same density (~300 fmol/mg) of rat or human β_3AR and human β_1- and β_2AR were used.

Adenylyl cyclase assay. Adenylyl cyclase activity was determined by the method of Salomon (15). Culture medium was removed and cells were washed in phosphate-buffered saline and then harvested in 25 mM HEPES (pH 8.0) buffer containing 2 mM $MgCl_2$ and 1 mM EDTA. Cells were homogenized and centrifuged at 48,000 × *g* for 15 min to obtain crude membranes. Membrane pellets were resuspended and used directly or were frozen at −80° until used. Freezing did not affect activity. Membranes (5–15 μg of protein) were preincubated with the specified drugs for 15 min at 4°, in a volume of 40 μl. Adenylyl cyclase reactions were initiated by addition of substrate mixture and were terminated after 30 min at 30°. The assay mixture containing the following constituents, in a final volume of 50 μl: 5 mM creatine phosphate, 50 Units/ml creatine phosphokinase, 25 mM Tris-acetate (pH 7.6), 10 mM magnesium acetate, 67 μM ATP, 0.5 mM cAMP, 1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 1 mM isobutylmethylxanthine, and 1–2 × 10^6 cpm of [α-^{32}]ATP (25–35 Ci/mmol). Concentration-response data were analyzed by nonlinear least squares regression analysis.

Radioligand binding assay. Competition studies were carried out in a manner similar to that described previously (14). Confluent cells were washed twice with phosphate-buffered saline and scraped into buffer containing 5 mM Tris (pH 7.40) and 2 mM EDTA, and the particulate suspension was washed twice by centrifugation at 38,000 × *g* and resuspension. Radioligand binding was carried out in 75 mM Tris (pH 7.40), 12.5 mM $MgCl_2$, 2 mM EDTA, 100 μM GTP buffer, using 300 pM ICYP and various concentrations of drugs as shown, in a final volume of 0.25 ml. Ascorbic acid was included in all assays at a final concentration of 1.0 mM, in order to protect agonists from oxidation. Membranes were incubated for 3 hr at 37°, and incubations were terminated by rapid vacuum filtration over glass fiber filters.

Materials. Reagents for tissue culture and adenylyl cyclase assays were from sources described previously (14). CGP was provided by Ciba-Geigy (Summitt, NJ), and BRL was from SmithKline-Beecham (Epsom, England). CHO cells were from the American Type Culture Collection.

Results

The full agonist ISO stimulated adenylyl cyclase activities to the same extent in membranes of CHO cells expressing the human and rat β_3AR (9.2 ± 1.0-fold versus 8.2 ± 0.7-fold, p = not significant). All three classical βAR agonists, ISO, NE, and EPI, were found to be agonists for both receptors, but clearly their potencies were low (K_{act} values in the micromolar range) for both species of β_3AR (Table 1). In contrast, we found that ISO was clearly more potent for the human β_1AR (K_{act} = 211 ± 45 nM, three experiments) and the human β_2AR (K_{act} = 55.3 ± 10.4 nM, three experiments), in parallel studies with membranes from CHO cells expressing these receptors. The rank order of potency for stimulation of adenylyl cyclase was the same for both species of β_3AR, i.e., ISO > NE > EPI (Table 1). As discussed below, the greater potency found here for ISO, compared with NE, for the human β_3AR is in contrast to what has been reported previously (5). Regarding intrinsic activities of catecholamines (compared with ISO), NE was the only full agonist, and only for the human β_3AR (Table 1).

Three noncatecholamine agonists that have been shown to interact with atypical β_3AR were also evaluated (Table 1). Clear differences in both efficacies and potencies were noted with these compounds between the human and rat β_3AR. BRL, which has been extensively studied *in vivo* with the rat atypical βAR (3), showed ~15-fold greater potency in stimulating adenylyl cyclase with the rat β_3AR, compared with the human β_3AR. Indeed, the relatively low potency (~6 μM) of BRL for the human β_3AR was about the same as that of ISO (~4 μM). On the other hand, BRL clearly displayed greater potency for the rat β_3AR than did ISO. Thus, BRL is a distinguishing compound for the rat but not for the human β_3AR. CGP showed the highest potencies of all compounds studied and in functional studies (Table 1) did not distinguish between the two species of receptors. Pindolol showed a relatively low potency for both β_3ARs. Differences in the intrinsic activities of these noncalectolamines were also found between the two β_3AR (Table 1). BRL was a full agonist for the rat β_3AR but had an intrinsic activity of 0.6 for the human receptor. CGP, on the other hand, was 2-fold more efficacious for the human, compared with the rat, β_3AR. Finally, pindolol was a weak agonist for the human β_3AR (0.36) and was virtually inactive with the rat receptor.

The results of radioligand binding studies (Table 2) closely paralleled those from adenylyl cyclase assays. (Pindolol was not included in this analysis because it was found to displace ICYP binding from nontransfected cells, presumably because of the similarity in structure of the two compounds). These results confirm that the β_3AR from both species has a low affinity for catecholamines. Other key findings delineated with the functional studies were also confirmed. Specifically, BRL had an affinity substantially greater than that of ISO for the

TABLE 1

Effects of agonists and partial agonists on adenylyl cyclase activity in membranes of CHO cells that express the human or rat β_3AR

Values are means ± standard errors (three to five experiments).

Agonist	K_{act}		Intrinsic activity (relative to ISO)	
	Human β_3AR	Rat β_3AR	Human β_3AR	Rat β_3AR
	μM			
ISO	4.0 ± 1.4	5.1 ± 1.1	1.0	1.0
NE	31 ± 11	7.5 ± 3.5	0.95 ± 0.04	0.80 ± 0.01
EPI	116 ± 16	68 ± 5	0.83 ± 0.01	0.75 ± 0.01
BRL	6.6 ± 16	0.44 ± 0.08	0.60 ± 0.01	1.0 ± 0.01
CGP	0.26 ± 0.03	0.23 ± 0.07	0.64 ± 0.05	0.31 ± 0.02
Pindolol	7.4 ± 0.8	30 ± 3.7	0.36 ± 0.01	0.10 ± 0.01

TABLE 2

Binding affinities of agonists and partial agonists in displacing ICYP from the human and rat β_3AR expressed in CHO cells

Values are means ± standard errors (four to six experiments).

Agonist	K_i	
	Human β_3AR	Rat β_3AR
	μM	
ISO	11.4 ± 3.3	38.6 ± 7.7
NE	23.1 ± 8.4	66.1 ± 15
EPI	245 ± 170	159 ± 51
BRL	1.46 ± 0.41	0.078 ± 0.010
CGP	0.028 ± 0.008	0.272 ± 0.156

rat β_3AR but an affinity similar to that of ISO for the human β_3AR. EPI had the lowest affinity of the catecholamines for either receptor. The potency rank order for catecholamines for inhibiting ICYP binding was ISO > NE > EPI, which is the same as that found for stimulation of adenylyl cyclase. Interestingly, CGP showed a high affinity for the human β_3AR, which was not the case for the rat receptor. This was the primary difference between the functional and the radioligand binding data comparing the two different β_3AR.

Discussion

Experiments with the cloned rat β_3AR (8, 9) indicate that its pharmacological properties are virtually identical to those of the atypical receptor in rat brown fat but differ in several respects from those originally reported and recently restated for the human receptor (5, 10, 11). Given that the β_3AR has been proposed as a potential therapeutic target for antidiabetes and antiobesity drugs (3, 16) and that these drugs are often developed using rodent models, we felt it was important to compare directly the pharmacological properties of the cloned human and rat receptors simultaneously under identical assay conditions. Our results indicate that the human and rat receptors are similar, in that they have low affinity for standard catecholamine agonists. As discussed below, this low affinity has important implications regarding the physiological role the β_3AR might play in adipocytes. In contrast to catecholamine agonists, our data have revealed numerous differences in the potencies and efficacies of noncatecholamine "atypical" agonists. These observations suggest that actions of atypical agonists in rodent models may not be representative of their effects in humans.

One important pharmacological property of the β_3AR is its affinity for NE, the presumed biological ligand. In the present experiments, radioligand binding assays, as well as adenylyl cyclase assays, indicate that NE has a low (micromolar) affinity for these receptors in both species. Furthermore, the close agreement between agonist potency (K_{act}) and affinity (K_i) indicates that there is little, if any, receptor reserve in adenylyl cyclase assays. This assertion is further supported by very recent binding data from the cloned rat β_3AR (9) and by adenylyl cyclase and binding data from the putative murine β_3AR in 3T3-442A cells (12). The low affinity of catecholamine agonists found in adenylyl cyclase assays of the cloned human and rodent β_3AR is also entirely consistent with work performed on intact or broken cell preparations of rodent adipocytes, which natively express the β_3AR (2, 8, 17–19).

These conclusions contrast sharply with those that might be drawn from studies using cAMP accumulation assays in cells that overexpress these receptors (5, 9, 11). For example, the initial characterization of the human β_3AR reported that NE, EPI, and ISO activated cAMP accumulation with approximately nanomolar potencies, which would seem to imply a high affinity interaction. It would appear, however, that cAMP accumulation assays may overestimate agonist affinity in cells overexpressing these receptors (e.g., Refs. 20 and 21). This may account for the 10–400-fold difference between K_{act} and K_i in the original report (5). Receptor overexpression, however, does not readily account for the lower (up to 80-fold) receptor binding affinities for various agonists at the human receptor observed in the present study (see also Ref. 12), for discrepancies in agonist potency order for the human receptor, or for the fact that CGP (which is more potent than and as efficacious as BRL) was reported to be inactive at the human receptor (5).

The low binding affinity of NE at the β_3AR has important implications regarding the physiological role this receptor plays in adipocytes. For example, rat adipose tissues express both β_1 and β_3AR (7, 22); however, NE has ~100-fold higher affinity for rat β_1AR than for rat β_3AR (23, 24). These observations indicate that β_1AR are likely to be most important at low physiological concentrations of catecholamines, whereas β_3AR might be active at higher concentrations, especially under conditions when β_1AR levels are low. Consistent with this hypothesis is the fact that metabolic and biochemical responses of adipose tissue that are evoked by physiological concentrations (i.e., <100 nM) (25) of NE are potently blocked by β_1AR-selective antagonists (or nonselective typical antagonists) (2, 7, 17, 18). In contrast, standard βAR antagonists are far less effective in blocking the effects of high concentrations of NE or in blocking β_3AR-selective agonists (1, 3, 7). Interestingly, the β_3AR appears to be particularly well suited for interactions with high concentrations of catecholamines. We have recently shown that the β_3AR fails to undergo functional desensitization during short term exposures (26) or down-regulation of receptor expression during long term exposures (27) to 100 μM ISO.

In contrast to catecholamine agonists, differences in agonist efficacy and/or potency were noted for each of the noncatecholamine atypical agonists tested. For example, BRL was the most potent atypical agonist of the rat β_3AR that was tested. Indeed, the potent metabotropic activity of BRL in rat tissues was one key finding that led to the discovery of the β_3AR (3). In contrast to the rat receptor, BRL was only a partial agonist of the human β_3AR and was about 15 times less potent in stimulating adenylyl cyclase activity. The low affinity and efficacy of BRL are consistent with its weak lipolytic activity in human adipocytes (28). CGP was previously reported to be inactive at the human β_3AR (5) and was considered to be one compound that would discriminate between human and rodent atypical receptors (28). Our data, however, indicate that CGP has high affinity at both β_3AR homologs and that the efficacy of CGP is actually greater at the human receptor. In this regard, CGP was as effective and 25 times more potent than BRL at the human β_3AR.

The reasons for the species differences in the sensitivity of β_3AR to atypical agonists are uncertain. The rat and human receptors are highly similar in the predicted transmembrane regions, and both contain conserved amino acids thought to be important in the binding of catecholamines to these proteins (29, 30). This homology probably accounts for the high degree of similarity between receptors in their sensitivity to catechol-

amines (Table 1). In contrast, atypical agonists, some of which are actually antagonists of the β_1- and β_2AR, probably interact with accessory binding sites that are distinct from those that form the catecholamine binding pocket. It is likely that there has been little selective pressure to maintain such accessory sites, compared with sites involved in the binding of endogenous ligands, and this could explain the species differences reported here. Finally, the data are also consistent with the notion that the cloned human β_3AR is not the homolog of the cloned rat β_3AR (6). This suggests that additional βAR subtypes may exist and have yet to be isolated.

To summarize, typical catecholamine agonists have a similar low affinity for both the rat and human β_3AR when these receptors are compared in parallel identical assays. In contrast, noncatecholamine atypical agonists, which are prototypes of those that might be developed as human therapeutic agents, differed with respect to potency and/or efficacy. These data indicate that the molecular actions of atypical βAR agonists differ markedly between species, and they resolve a number of important discrepancies in the current literature regarding the β_3AR.

Acknowledgments

We thank James Granneman for supplying the rat β_3AR, Lynn Chesnut and Kathy Bendt for technical assistance, and Shirley McLean for typing the manuscript.

References

1. Bojanic, D., J. D. Jansen, S. R. Nahorski, and J. Zaagsma. Atypical characteristics of the β-adrenoceptor mediating cyclic AMP generation and lipolysis in the rat adipocyte. *Br. J. Pharmacol.* **84**:131–137 (1985).
2. Levin, B. E., and A. C. Sullivan. *Beta*-1 receptor is the predominant *beta*-adrenoreceptor on rat brown adipose tissue. *J. Pharmacol. Exp. Ther.* **236**:681–688 (1986).
3. Arch, J. R. S., A. T. Ainsworth, M. A. Cawthorne, V. Piercy, M. W. Sennitt, V. E. Thody, C. Wilson, and S. Wilson. Atypical *beta*-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature (Lond.)* **309**:163–165 (1984).
4. Holloway, B. R., R. Howe, B. S. Rao, D. Stribling, R. M. Mayers, M. G. Briscoe, and J. M. Jackson. ICI D7114, a novel selective β-adrenoceptor agonist, selectively stimulates brown fat and increases whole-body oxygen consumption. *Br. J. Pharmacol.* **104**:97–104 (1991).
5. Emorine, L. J., S. Marullo, M.-M. Breind-Sutren, G. Patey, K. Tate, C. Delavier-Kluthko, and A. D. Strosberg. Molecular characterization of the human $beta_3$ adrenergic receptor. *Science (Washington D. C.)* **245**:1118–1121 (1989).
6. Zaagsma, J., and S. R. Nahorski. Is the adipocyte β-adrenoreceptor a prototype for the recently cloned atypical "β_3-adrenoreceptor"? *Trends Pharmacol. Sci.* **11**:3–7 (1990).
7. Granneman, J. G. Norepinephrine and BRL 37344 stimulate adenylate cyclase by different receptors in brown adipose tissue. *J. Pharmacol. Exp. Ther.* **254**:508–513 (1990).
8. Granneman, J. G., K. N. Lahners, and A. Chaudhry. Molecular cloning and expression of the rat $beta_3$ adrenergic receptor. *Mol. Pharmacol.* **40**:895–899 (1991).
9. Muzzin, P., J.-P. Revelli, F. Kuhne, J. D. Gocayne, W. R. McCombie, J. C. Venter, J.-P. Giacobino, and C. M. Fraser. An adipose tissue-specific β-adrenergic receptor. *J. Biol. Chem.* **266**:24053–24058 (1991).
10. Tate, M., M.-M. Briend-Sutren, L. J. Emorine, C. Devalier-Klutchko, S. Marullo, and A. D. Strosberg. Expression of three β-adrenergic-receptor subtypes in transfected Chinese hamster ovary cells. *Eur. J. Biochem.* **196**:357–361 (1991).
11. Nahmais, C., N. Blin, J.-M. Elalouf, M. G. Mattei, A. D. Strosberg, and L. J. Emorine. Molecular characterization of the β_3-adrenergic receptor: relationship with the atypical receptor of adipocytes. *EMBO J.* **10**:3721–3727 (1991).
12. Fevé, B., L. J. Emorine, F. Lasnier, N. Blin, B. Baude, C. Nahmais, A. D. Strosberg, and J. Pairault. Atypical β-adrenergic receptor in 3T3-F442A adipocytes. *J. Biol. Chem.* **266**:20329–20336 (1991).
13. Liggett, S. B., and D. A. Schwinn. Multiple potential regulatory elements in the 5′ flanking region of the human β_3-adrenergic receptor. *DNA Sequence* **2**:61–63 (1991).
14. Greene, S., B. Holt, and S. B. Liggett. β_1- and β_2-adrenergic receptors display subtype specific coupling to G_s. *Mol. Pharmacol.* **41**:889–893 (1992).
15. Salomon, U. Adenylate cyclase assay. *Adv. Cyclic Nucleotide Res.* **10**:35–55 (1979).
16. Arch, J. R. S. The brown adipocyte *beta*-adrenoceptor. *Proc. Nutr. Soc.* **48**:215–223 (1989).
17. Bukoweicki, L., N. Follea, A. Paradis, and A. Collet. Stereospecific stimulation of brown adipocyte respiration by catecholamines via *beta*-1 adrenoreceptors. *Am. J. Physiol.* **238**:E563–E569 (1980).
18. Atglé, C., A. Marette, and L. J. Bukoweicki. Norepinephrine and BRL 37344 stimulate thermogenesis in rat brown adipose tissue via different receptors. *Int. J. Obesity* **15**:52–56 (1991).
19. Hollenga, C., F. Brouwer, and J. Zaagsma. Relationship between lipolysis and cyclic AMP generation mediated by atypical β-adrenoreceptors in rat adipocytes. *Br. J. Pharmacol.* **102**:577–580 (1991).
20. Bouvier, M., M. Hnatowich, S. Collins, B. K. Kobilka, A. Deblasi, R. J. Lefkowitz, and M. G. Caron. Expression of a human cDNA encoding the β_2-adrenergic receptor in Chinese hamster fibroblasts (CHW): functionality and regulation of the expressed receptors. *Mol. Pharmacol.* **33**:133–139 (1988).
21. George, S. T., M. Berrios, J. R. Hadcock, H.-Y. Wang, and C. C. Malbon. Receptor density and cAMP accumulation: analysis in CHO cells exhibiting stable expression of a cDNA that encodes the $beta_2$-adrenergic receptor. *Biochem. Biophys. Res. Commun.* **150**:665–672 (1988).
22. Granneman, J. G., and K. N. Lahners. Differential adrenergic regulation of β_1 and β_3-adrenoreceptor messenger ribonucleic acids in adipose tissues. *Endocrinology* **130**:109–114 (1992).
23. Machida, C. A., J. R. Brunzow, R. P. Searles, H. Van Thol, B. Tester, K. A. Neve, P. Teal, V. Nipper, and O. Civelli. Molecular cloning and expression of the rat β_1 receptor gene. *J. Biol. Chem.* **265**:12960–12965 (1990).
24. Chaudhry, A., K. N. Lahners, and J. G. Granneman. Perinatal changes in the coupling of $beta_1$ and $beta_3$-adrenergic receptors to brown fat adenylyl cyclase. *J. Pharmacol. Exp. Ther.* **261**:633–637 (1992).
25. Foster, D. O., F. Depocas, G. Zaror-Behrens, M. L. Frydman, and S. Lacelle. Effects of rate of blood flow on fractional extraction and on uptake of infused noradrenaline by brown adipose tissue *in vivo*. *Can. J. Physiol. Pharmacol.* **58**:1212–1220 (1980).
26. Liggett, S. B., D. A. Schwinn, and R. J. Lefkowitz. Structural basis for receptor subtype specific desensitization revealed by a chimeric β_3/β_2-adrenergic receptor. *Clin. Res.* **40**:252A (1992).
27. Thomas, R. F., B. D. Holt, D. A. Schwinn, and S. B. Liggett. Long term agonist exposure induces novel up-regulation of β_3-adrenergic receptor expression via multiple cAMP response elements. *Proc. Natl. Acad. Sci. USA* **89**:4490–4494 (1992).
28. Emorine, L. J., B. Feve, J. Pairault, M.-M. Briend-Sutren, S. Marullo, C. Devalier-Klutchko, and A. D. Strosberg. Structural basis for the functional diversity of β_1, β_2 and β_3-adrenergic receptors. *Biochem. Pharmacol.* **41**:853–859 (1991).
29. Strader, C. D., I. S. Sigal, R. B. Register, M. R. Candelore, E. Rands, and R. A. F. Dixon. Identification of residues required for ligand binding to the β-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **84**:4384–4388 (1987).
30. Strader, C. J., M. R. Candelore, W. S. Hill, I. S. Sigal, and R. A. F. Dixon. Identification of two serine residues involved in agonist activation of the β-adrenergic receptor. *J. Biol. Chem.* **264**:13572–13578 (1989).

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