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β1-Adrenergic Receptors Mediate β3-Adrenergic-Independent Effects of CGP 12177 in Brown Adipose Tissue

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ABSTRACT

CGP 12177 is a β-adrenergic receptor (AR) ligand that has been used to characterize the β3-AR and the putative β4-AR. The ability of CGP 12177 to activate β1-AR when overexpressed in vitro and the presence of β1-AR in tissues expressing putative β4-AR prompted us to investigate the actions of CGP 12177 at recombinant and natively-expressed β-AR. CGP 12177 potently activated recombinant rat and human β1-AR expressed in Chinese hamster ovary cells. This activation, like that of putative β4-AR, was resistant to blockade by selective and nonselective β-AR antagonists. Brown fat has been proposed to contain β4-AR, as evidenced by the presence of CGP 12177-mediated thermogenesis in mice lacking β3-AR. Therefore, the identity of the receptors mediating CGP 12177 responses in brown fat was examined using wild-type mice and mice lacking β1-AR or β3-AR. In wild-type mice, CGP 12177 activated adenylyl cyclase via high- and low-affinity sites. The high-affinity site, but not the low-affinity site, was blocked by CGP 20712 with potency indicating an interaction with β1-AR. Moreover, the high-affinity site was absent in mice lacking β1-AR. In contrast, the low-affinity, CGP 20712-resistant activation by CGP 12177 was absent in mice lacking β3-AR. Rather, activation occurred exclusively through the high-affinity, CGP 20712-sensitive site. These data indicate that the actions of CGP 12177 in brown fat that have been attributed to novel β-AR (i.e., β4-AR) are mediated via an atypical interaction with β1-AR.

CGP 12177 is an aryloxypropanolamine that was originally developed as a high-affinity β1/2-adrenergic receptor (AR) ligand (Staehelin et al., 1983). Although CGP 12177 is a potent antagonist of β1/2-AR, it also exhibits sympathomimetic activity, suggesting partial agonist activity at these receptors or interaction with additional receptor types. CGP 12177 was found subsequently to activate brown fat thermogenesis and adenylyl cyclase through a mechanism involving β3-AR (Mohell and Dicker, 1989; Granneman and Whitty, 1991; Granneman et al., 1993). Although many of its agonist actions have been attributed to activation of β3-AR, the differential activities of CGP 12177 and phenethanolamine agonists in stimulating certain cardiovascular and metabolic responses indicate that CGP 12177 might interact with receptor sites other than the β3-AR (Kaumann and Molenaar, 1996, 1997; Galitzky et al., 1997). In fact, the unique pharmacological profile of CGP 12177 led to the proposal of an additional atypical β-AR subtype, termed the β4-AR, whose existence recently gained support from studies demonstrating that CGP 12177 activates brown adipose and cardiac tissue responses in mice lacking the β3-AR (Ito et al., 1998; Kaumann et al., 1998; Preitner et al., 1998).

CGP 12177-induced responses attributed to putative β4-AR have been observed in tissues, such as heart and adipose, that express high levels of β1-AR. Recently, CGP 12177 was found to activate β1-AR when the receptor was overexpressed in cultured cells (Pak and Fishman, 1996), raising the possibility that β1-AR might be involved in mediating putative β4-AR responses. The relevance of these observations to the classification of atypical β-AR (i.e., β3-AR and β4-AR), however, was unclear. First, it was uncertain whether CGP 12177-mediated activation of β1-AR met the pharmacological criteria used to define β4-AR. Second, it was unknown whether CGP 12177-mediated activation required β1-AR overexpression, as suggested by Pak and Fishman (1996), or could occur in tissues that natively express the receptor. Therefore, in this study, we characterized pharmacological properties of CGP 12177 with recombinant rat and human β1-AR. We confirm that CGP 12177 is a potent agonist of β1-AR and demonstrate that this activity, as in that at putative β4-AR, is resistant to blockade by standard β-AR antagonists. Analysis of interactions with recombinant β1- and β3-AR allowed the design of pharmacological conditions to test whether CGP 12177 activates β1-AR in brown fat, a tissue thought to express the putative β4-AR (Galitzky et al., 1997; Ito et al., 1998; Preitner et al., 1998). When performed on tissues from mice lacking β1- or β3-AR, this pharmacological analysis provided compelling data indicating that

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ABBREVIATIONS: AR, adrenergic receptor(s); CHO, Chinese hamster ovary; KO, knock out.

β1-AR mediate most, if not all, β3-AR-independent effects of CGP 12177 on brown fat adenylyl cyclase activity.

Experimental Procedures

Materials. Materials for adenylyl cyclase assays were obtained from sources described previously (Chaudhry and Granneman, 1991). Ham's F-12 medium was purchased from Irvine Scientific (Santa Anna, CA). Penicillin/streptomycin and geneticin were obtained from Life Technologies (Gaithersburg, MD). Drugs were obtained from the following sources: (−)-isoproterenol bitartrate, (−)-propranolol hydrochloride (Sigma Chemical, St. Louis, MO); CGP 12177, CGP 20712A (Research Biochemical Inc., Natick, MA). All other chemicals were of reagent grade.

Cell Culture and Transfections. Chinese hamster ovary (CHO) cells were grown in Ham's F-12 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100,000 U/L penicillin, and 100 mg/L streptomycin in a humidified atmosphere of 5% CO_2. Cells were harvested at about 90% confluence. Rat and human β1-AR cDNAs were expressed in CHO cells as described previously (Chaudhry et al., 1992). The human β1-AR cDNA was a gift from Dr. S. Liggett (University of Cincinnati, Cincinnati, OH). Cells were washed twice with PBS, pH 7.4, then lysed in a hypotonic homogenization buffer containing 25 mM HEPES, pH 8.0, 2 mM $MgCl_2$, 1 mM EDTA, and 10 μg/ml leupeptin. Cell membranes were collected with the use of a rubber policeman and pelleted by centrifugation at 48,000*g* for 15 min at 4°C. Membranes were stored at −80°C before being resuspended in homogenization buffer for use in experiments.

Animals. Mice deficient in the expression of β3-AR (β3-AR KO) or β1-AR (β1-AR KO) were provided, respectively, by Dr. Bradford Lowell (Massachesetts General Hosptial, Brookline, MA) and Dr. Brian Kobilka (Stanford University, Stanford, CA), and bred at Wayne State University. The β3-AR KO mice were generated on the FVB/n strain (Taconic Farms, Germantown, NY), which served as control subjects. The β1-AR KO mice were derived from a mixed background of 129Sv, C57Bl6/J, and DBA2/J. Wild-type control subjects of either sex were age-matched mice of the same strain and background. Animals were used at 1 to 2 months of age. Two hours before euthanasia, mice were injected with reserpine (5 mg/kg, i.p.) to deplete endogenous norepinephrine and thereby reduce basal adenylyl cyclase activity (Granneman, 1990). Mice were sacrificed by cervical dislocation and tissues were rapidly dissected and frozen at −80°C until used. All procedures were approved by the Institutional Animal Investigation Committee in strict accordance with the *NIH Guide for the Care and Use of Laboratory Animals*.

Adenylyl Cyclase Assay. Adenylyl cyclase activity was determined by a modification (Granneman et al., 1991) of the method of Salomon (1979). Briefly, membranes (5 to 20 μg of protein) were incubated with agonists at 4°C in the presence or in the absence of various β-AR antagonists for 20 to 30 min. The adenylyl cyclase reaction was initiated by addition of the substrate mix and carried out for 10 to 20 min at 30°C. The reactions were terminated by the addition of 20 μl of 2.2 N HCl. The contents of the substrate mix and chromatographic separation of cAMP have been described previously (Chaudhry and Granneman, 1991, 1994).

Kinetic parameters of concentration-response curves were determined using GraphPAD Prism software (San Diego, CA). β-AR antagonist affinities (K_B) at rat β1-AR were analyzed using a Schild plot (Arunlakshana and Schild, 1959). K_B values at the human β1-AR were calculated from shift in agonist concentration-response curve produced by a single concentration of antagonist according to the equation $K_B = [B]/DR - 1$, where [B] is antagonist concentration and DR is the ratio of EC_{50} in the presence and absence of antagonist. Antagonist affinities (IC_{50}) were estimated from antagonist

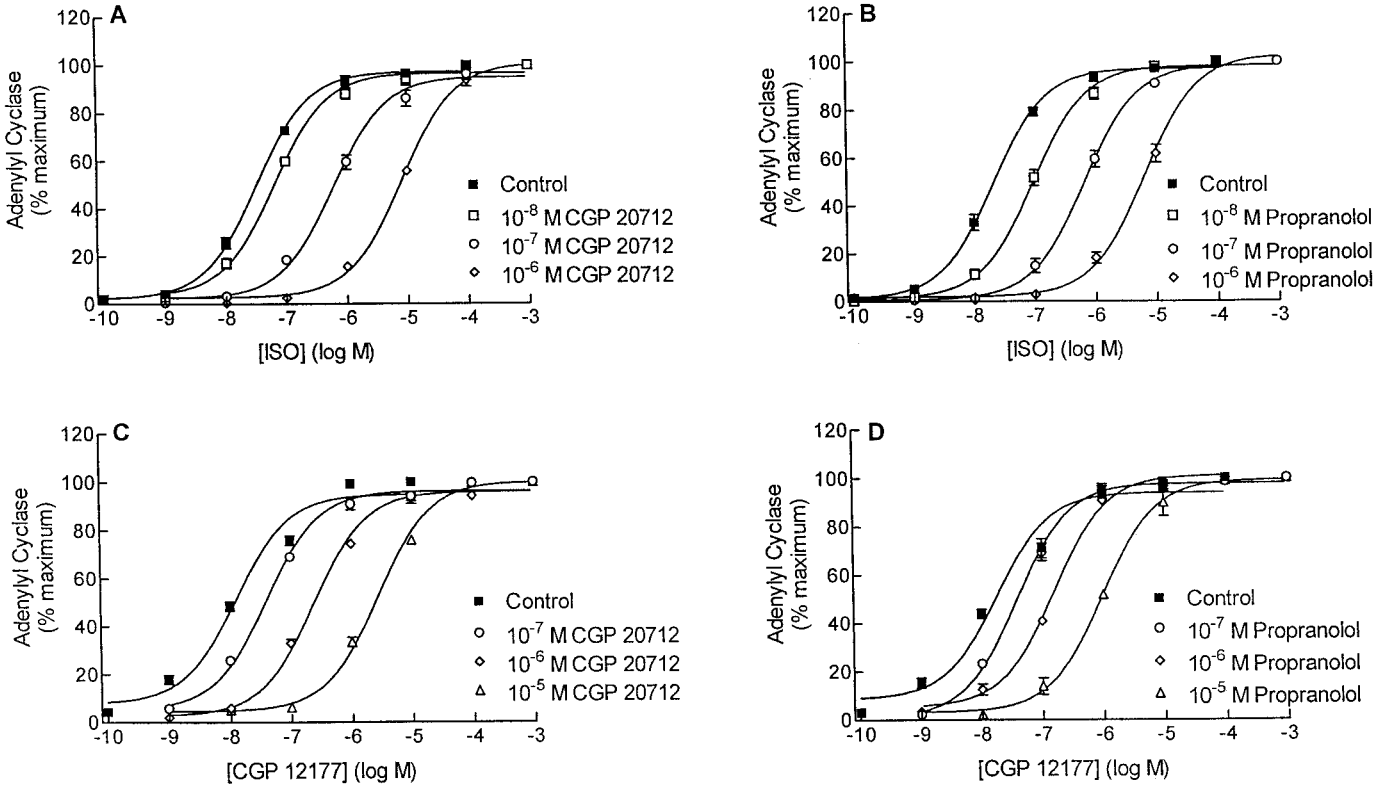

Fig. 1. Antagonism of isoproterenol- (ISO; A and B) and CGP 12177-induced (C and D) activation of rat β1-AR by increasing concentrations of the β1-AR-selective antagonist CGP 20712 (A and C) and nonselective antagonist propranolol (B and D) in CHO cells. Adenylyl cyclase activation is expressed as a percentage of the maximal response to isoproterenol or CGP 12177. Schild regression of antagonism of isoproterenol and CGP 12177-induced activation of β1-AR by CGP 20712 and propranolol gave slope values that were not significantly different from unity. Values are presented as means ± S.E. of three to six experiments.

inhibition curves with Gaddum's formula (Gaddum, 1937). Kinetic parameters were estimated for each independent experiment, then averaged for presentation. Values presented are means ± S.E. Student's t test was used to evaluate differences between means and critical values of $P < .05$ were judged significant.

Results

Pharmacological Analysis of CGP 12177 at Recombinant β1-AR. CGP 12177 activated adenylyl cyclase in CHO cell membranes expressing rat and human β1-AR (Figs. 1 and 2), but was inactive in untransfected cells (not shown). CGP 12177 was nearly equipotent to isoproterenol but was nonetheless a partial agonist (Table 1). The intrinsic activity of CGP 12177 at β1-AR and β3-AR is similar, whereas the compound is 20 to 50 times more potent at stimulating β1-AR (Granneman et al., 1991).

Antagonist potencies are widely used to define the sites of agonist-receptor interaction. Differences in the potencies of antagonists to block isoproterenol and CGP 12177 have led to the proposal of a novel receptor, the β4-AR. Therefore, we examined the potencies of standard β-AR antagonists to block activation of rat β1-AR by CGP 12177 and the reference catecholamine agonist isoproterenol. The concentration-response curves to CGP 12177 and isoproterenol were shifted rightward in the presence of increasing concentrations of the β1-AR-selective antagonist CGP 20712 (Fig. 1). Calculation of the K_B value of CGP 20712 indicated that CGP 12177 activation of adenylyl cyclase was about 6 times ($P < .05$) more resistant to blockade than was catecholamine activation. The discrepancy between antagonist potencies was more dramatic for propranolol, which was more than 40-fold less effective ($P < .05$) in blocking stimulation by CGP 12177 versus isoproterenol (Fig. 1; Table 1).

CGP 12177-induced activation of human β1-AR was also more resistant to blockade by CGP 20712 and propranolol than isoproterenol (Fig. 2). Calculation of K_B indicated that an 18-fold greater concentration ($P < .05$) of CGP 20712A was required to block activation of β1-AR by CGP 12177 versus isoproterenol (Fig. 2). Similarly, a 26-fold higher concentration ($P < .01$) of propranolol was required to block activity stimulated by CGP 12177 versus isoproterenol (Fig. 2; Table 1).

Analysis of CGP 12177 Activation of Brown Fat Adenylyl Cyclase in Wild-Type and β-AR-Deficient Mice. The effects of CGP 12177 were first assessed in brown adipose tissue membranes from wild type FVB/n and mixed 129Sv, C57Bl6/J, DBA2/J mice (Fig. 3, top). Stimulation of brown fat adenylyl cyclase by CGP 12177 was highly similar in both sets of wild-type mice. Activation of adenylyl cyclase occurred over more than 4 log units, suggesting that CGP 12177 interacts with more than one receptor site. Detailed analysis of the concentration-response curves clearly indicated biphasic stimulation of adenylyl cyclase that could be modeled by a two-site, mass action equation (Table 2). In FVB/n mice, the high-affinity component constituted about 40% of the total response and had a K_{act} value of 13 nM, whereas the low-affinity component had a K_{act} value that was more than 50-fold greater. Similar estimates were obtained in wild-type β1-AR mice, which exhibited a high-affinity (7 nM) component, accounting for about 40% of the total response, and a low-affinity component (700 nM), constituting the remaining 60%.

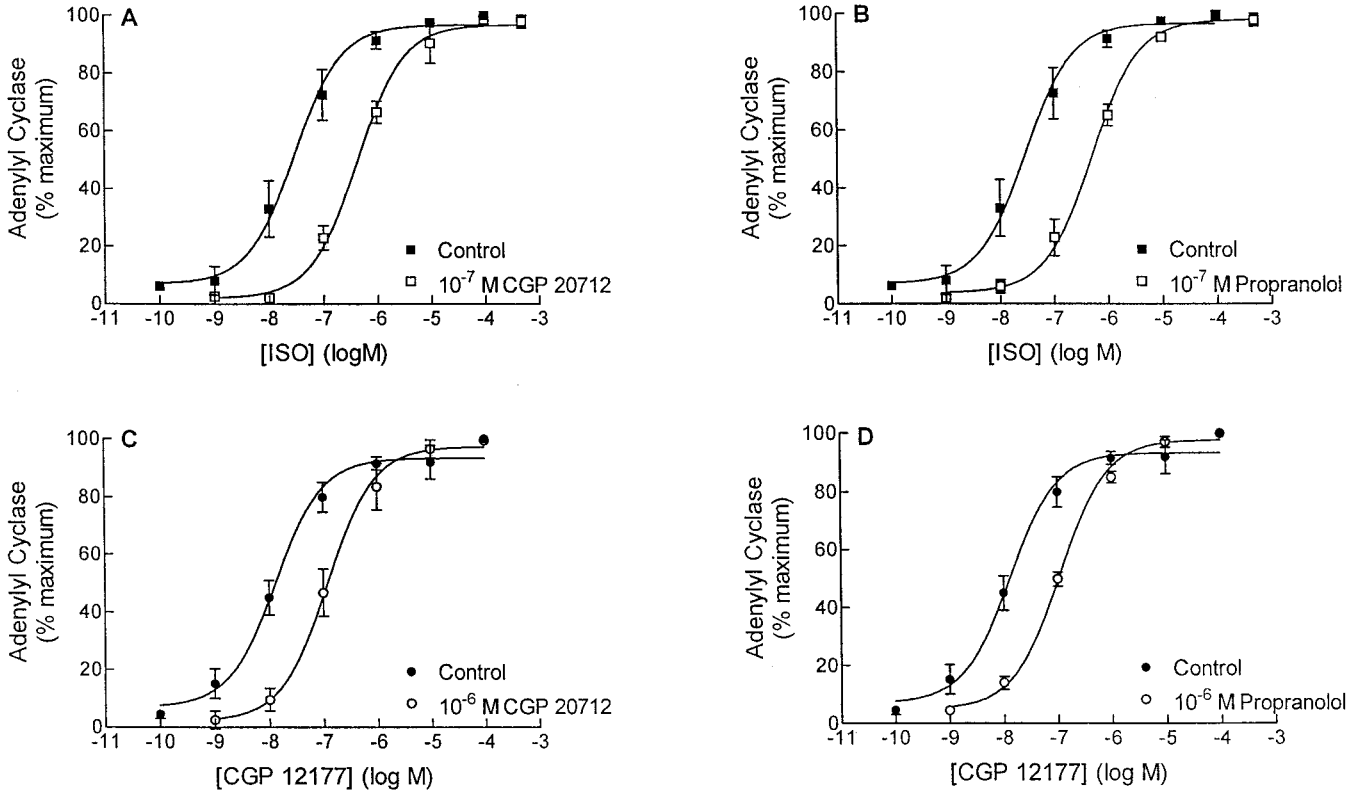

Fig. 2. Antagonism of isoproterenol- (ISO; A and B) and CGP 12177-induced (C and D) activation of human β1-AR by the β1-AR selective antagonist CGP 20712 (A and C) and nonselective antagonist propranolol (B and D) in CHO cells. Adenylyl cyclase activation is expressed as a percentage of the maximal response to isoproterenol or CGP 12177. Values are presented as means ± S.E. of three experiments.

Based on past and present pharmacological analysis of recombinant β-AR subtypes (Granneman et al., 1991, 1993), we hypothesized that the high-affinity component was mediated by β1-AR, whereas the low-affinity component represented β3-AR. To test this, we examined the effects of CGP 20712, a highly selective β1-AR receptor antagonist that blocks CGP 12177 agonist activity at the cloned β1-AR (Figs. 1 and 2), but is inactive at the cloned β3-AR (Granneman et al., 1991). CGP 20712 (1 μM) strongly antagonized the high-affinity component but had little if any effect on the low-affinity component (Fig. 3, A and B). Indeed, in the presence of CGP 20712, activation of brown fat adenylyl cyclase by CGP 12177 was monophasic and occurred with potency similar to the low-affinity site found in untreated membranes (Table 2). Although antagonist affinities are difficult to determine precisely in tissues containing a mixture of subtypes, the pK_B value of CGP 20712 at the high-affinity site was estimated to be ≥ 7.5, whereas the pK_B at the low-affinity site was ≤ 6.0. These data provide strong pharmacological evidence that β1-AR and β3-AR mediate the high- and low-affinity components, respectively, of CGP 12177-induced adenylyl cyclase activation.

The availability of mice lacking β1-AR (Rohrer et al., 1996) and β3-AR (Susulic et al., 1995) allowed us to directly assess involvement of β-AR subtypes in CGP 12177-mediated brown fat activation. In mice lacking β3-AR, CGP 12177 stimulated brown fat adenylyl cyclase to a degree similar to that in wild-type mice (Fig. 3C). In contrast to wild type mice, however, this activation seemed to be mediated by a single class of receptors having a high affinity for CGP 12177 (Table 2). The potency of CGP12177 at this high-affinity site (7 nM) was very similar to the high-affinity site detected in wild-type mice. In contrast, the low-affinity (K_{act} = 700 nM) component of CGP 12177-mediated activation seen in FVB/n mice was absent in the β3-AR KO mice, fulfilling the prediction that this component is mediated by the β3-AR.

CGP 20712 (1 μM) produced a strong, parallel rightward shift in the CGP 12177 concentration-response curve in brown fat membranes from β3-AR KO mice. Based on the magnitude of the dextral shift, the affinity of CGP 20712 was calculated to be 38 ± 10 nM (n = 5). This value is consistent with the potency of CGP 20712 in blocking the high-affinity site seen in wild-type mice. Furthermore, this estimate agrees closely with values obtained at recombinant β1-AR

TABLE 1
Pharmacological parameters of rat and human β1-AR activation by isoproterenol and CGP 12177 in CHO cells

	EC_{50} value	Intrinsic activity	K_B values	
			CGP 20712A	Propranolol
	nM		*nM*	
Rat β1-AR				
Isoproterenol	25.9 ± 3.6	1.00	7.3 + 1.2	2.5 ± 0.3
CGP 12177	15.4 ± 2.6	0.43 ± 0.1	44.8 ± 9.8	104.3 ± 46.9
Human β1-AR				
Isoproterenol	37.0 ± 19.9	1.00	7.6 ± 2.6	7.0 ± 3.1
CGP 12177	15.2 ± 5.7	0.45 ± 5.7	121.7 ± 28.4	192.3 ± 88.4

EC_{50}, concentration of agonist inducing half-maximal adenylyl cyclase activation; intrinsic activity, maximum effect of agonists as compared to maximum effect of isoproterenol. Values are means ± S.E. of n = 3 experiments. K_B values were calculated as detailed under *Experimental Procedures*.

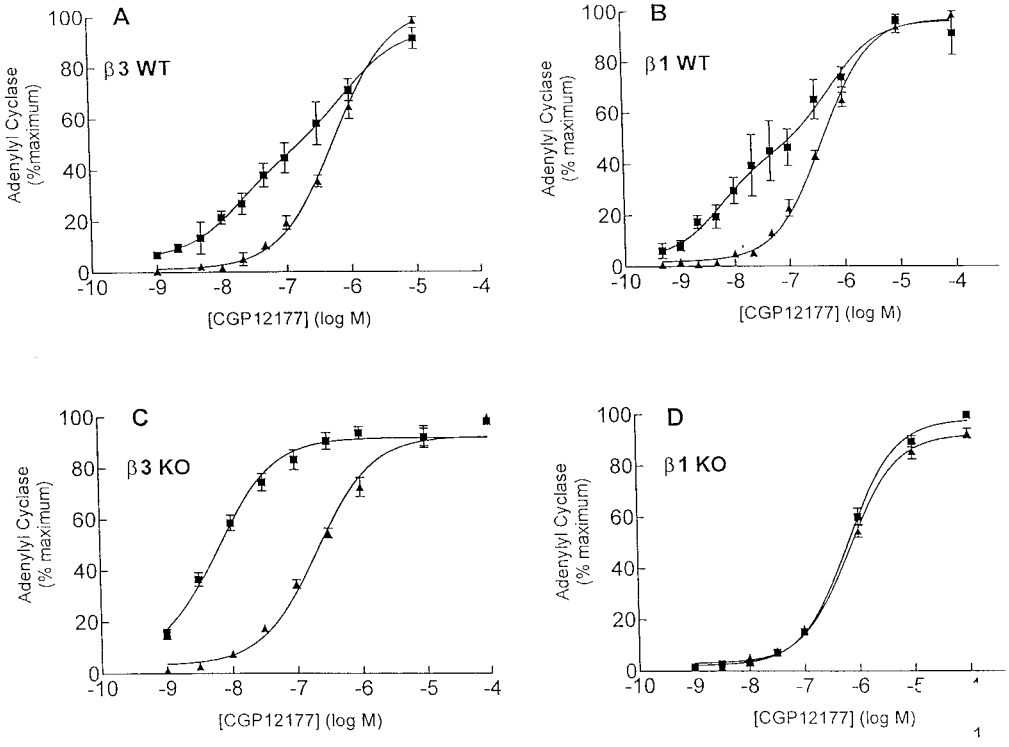

Fig. 3. Activation of adenylyl cyclase by CGP 12177 in brown fat membranes of wild-type and β-AR KO mice. Adenylyl cyclase activity was determined in the absence (■) and presence (▲) of 1 μM CGP 20712. A, β3 wild-type (WT), control FVB/n mice. B, β1-WT, control 129Sv, C57Bl6/J, DBA2/J mice. C: β3-KO, β3-AR-KO mice. D, β1-KO, β1-AR-KO mice. Average basal activities and maximal adenylyl cyclase activities (in picomoles per minute per milligram of protein) were: β3-WT, 44 ± 8 and 142 ± 16; β3-KO, 89 ± 9 and 236 ± 36; β1-WT, 63 ± 13 and 173 ± 20; β1-KO, 38 ± 5 and 204 ± 33. Values are means ± S.E. for three to five experiments.

expressed in CHO cells (Figs. 1 and 2). In contrast, ICI 118,551, a high-affinity β2-AR-selective antagonist, exhibited very low potency in blocking CGP 12177 ($pK_B > 6$, not shown), indicating that β2-AR are not involved in the response.

We next examined the stimulation of adenylyl cyclase in brown fat membranes of β1-AR knockout (KO) mice. CGP 12177 stimulated adenylyl cyclase with a potency that corresponded closely with the low-affinity site seen in wild-type control mice (Fig. 3, B and D; Table 2). Moreover, CGP 20712 (1 μM) failed to block this activity. These data are consistent with activation solely via β3-AR. Perhaps more to the point, these data clearly demonstrate that the high-affinity, CGP 20712-sensitive activation by CGP 12177 requires expression of the β1-AR.

Analysis of recombinant β1-AR indicated that β-AR antagonists are significantly less potent in blocking CGP 12177 versus catecholamine agonists (Figs. 1 and 2). To determine whether this behavior holds for natively expressed β1-AR, we examined the inhibition of isoproterenol- and CGP 12177-stimulated adenylyl cyclase activity by selective and nonselective β-AR antagonists. Brown fat membranes of β3-AR KO mice were stimulated with isoproterenol in the absence or presence of a fixed concentration of propranolol (Fig. 4). A 10-fold greater concentration of propranolol was required to produce and equivalent rightward shift of the concentration-response curve for CGP 12177 versus isoproterenol. Calculation of the K_B value indicated that CGP 12177-mediated activation of adenylyl cyclase was significantly ($P < .01$) more resistant to propranolol blockade than was isoproterenol-mediated activation (142 ± 26 nM versus 13.6 + 3.3 nM, $n = 3$).

We also examined inhibition of isoproterenol- and CGP 12177-mediated effects by β-AR subtype-selective antagonists in brown fat membranes from β3-AR KO mice (Fig. 5). The β1-AR antagonist CGP 20712 blocked isoproterenol-stimulated activity (IC_{50} = 31 ± 6 nM, $n = 3$), but this activity was much less potently antagonized by the β-AR antagonist ICI 118,551. In contrast to isoproterenol, CGP 20712 was 10-fold less potent (IC_{50} = 342 ± 11, $n = 3$) in suppressing CGP 12177-stimulated activity. Similar results were obtained with propranolol (not shown). The K_i values of CGP 20712 calculated from these inhibition curves agree closely with the K_B values obtained above. ICI 118,551 displayed very low potency and efficacy in blocking CGP 12177.

TABLE 2
Pharmacological parameters of adenylyl cyclase activation by CGP 12177 in brown fat membranes of control and βAR-KO mice.

Tissue	K_{act} CGP 12177			K_B CGP 20712
	K_H	K_L	%H	
		nM		*nM*
β3-AR-WT	13 ± 4	705 ± 243	41 ± 5	
β3-AR-KO	7 ± 2	ND	100	38 ± 10
β1-AR-WT	5 ± 2	687 ± 145	41 ± 10	
β1-AR-KO	ND	683 ± 102	0	>1,000

ND, not detected; %H, percentage of total response mediated by high-affinity sites; WT, wild-type. Values are mean ± S.E.; n = 3–5.

Discussion

CGP 12177 was developed originally as a β1/2-AR antagonist but has since been used to activate "atypical" β-AR (i.e., the β3-AR and the putative β4-AR). Although CGP 12177 clearly activates β3-AR, the finding that CGP 12177 activates certain cardiovascular and metabolic responses under conditions that highly effective phenethanolamine β3-AR agonists do not strongly indicates that there are additional receptor sites for CGP 12177 (Galitzky et al., 1997; Kaumann and Molenaar, 1997). Pak and Fishman (1996) first reported that CGP 12177 activates β1-AR, but not β2-AR, in transfected cells. However, because this effect was thought to require receptor overexpression, the relevance of this observation to the function of natively expressed β1-AR was specifically discounted. The correlation of β1-AR expression with CGP 12177 responsiveness in tissues lacking significant β3-AR expression, however, prompted us to further characterize the interaction of this compound with recombinant β1-AR and mice deficient in β1-AR or β3-AR subtypes.

The use of CGP 12177 to define β4-AR assumes that this compound possesses only antagonist activity at β1-AR and β2-AR. However, we verified the observation of Pak and Fishman (1996) that CGP 12177 is indeed a potent partial agonist of recombinant rat and human β1-AR. In this regard, it is interesting to note that although CGP 12177 is a partial agonist of both β1-AR and β3-AR, this compound is nearly 20 times more potent at β1-AR (Granneman et al., 1991, 1993). The pharmacological criteria used to define the β4-AR in-

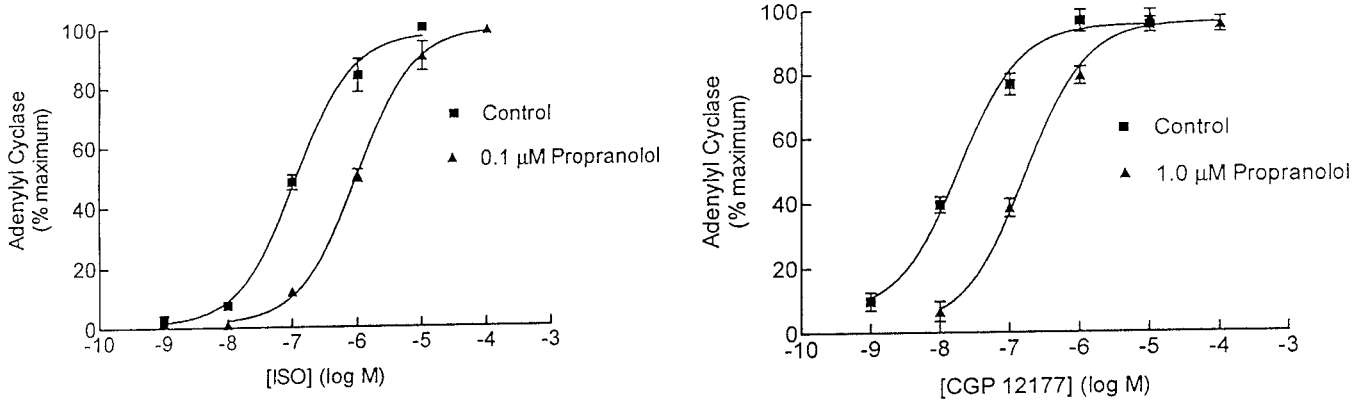

Fig. 4. Inhibition of isoproterenol- (ISO) and CGP 12177-stimulated adenylyl cyclase activity in brown fat membranes of β3-AR-KO mice by propranolol. Membranes were stimulated by increasing concentrations of isoproterenol (ISO) or CGP 12177 in the absence or presence of 0.1 μM or 1.0 μM propranolol. Intrinsic activity of CGP 12177 (relative to isoproterenol) was 0.25 ± 0.01. Values are means ± S.E. for three experiments.

clude resistance to β-AR blockade and lack of activation by β3-AR selective phenethanolamine agonists. The present work demonstrates that activation of rat and human β1-AR by CGP 12177 is 10 to 20 times more resistant to selective and nonselective β-AR blockade. Lastly, we have shown previously that β3-AR-selective phenethanolamine agonists fail to stimulate β1-AR (Granneman et al., 1998). Thus, CGP 12177 responses at recombinant rat and human β1-AR fulfill the criteria set for distinguishing the putative β4-AR.

Pharmacological analysis of recombinant receptors allowed the design of experiments to determine whether β1-AR mediate CGP 12177 agonist effects in brown fat, a tissue that has been proposed to contain β4-AR (Galitzky et al., 1997; Ito et al., 1998; Preitner et al., 1998). As predicted from the differential affinity for cloned β1-AR and β3-AR, CGP 12177 was found to activate brown fat adenylyl cyclase of control mice via interactions with high- and low-affinity receptor sites. Furthermore, the β1-AR antagonist CGP 20712 antagonized the high-affinity component but had no effect on the low-affinity component. Moreover, the potency of CGP 20712 in suppressing the high-affinity component corresponded closely to values obtained at the recombinant β1-AR. Together, these data provide strong pharmacological evidence that β1-AR and β3-AR mediate, respectively, the high and low affinity activation of brown fat adenylyl cyclase by CGP 12177.

These conclusions were further substantiated using β1-AR- and β3-AR KO mice. Brown adipose tissue membranes from β3-AR KO mice specifically lacked low-affinity, CGP 20712-insensitive activation of adenylyl cyclase by CGP 12177. Rather, activation of adenylyl cyclase occurred through a single site whose affinity was identical with the high-affinity component seen in control membranes. As expected, this site was potently antagonized by CGP 20712. Consistent with work using recombinant β1-AR, the inhibitory potencies of CGP 20712 and propranolol were significantly lower for CGP 12177 versus isoproterenol, further demonstrating that β1-AR mediate the effects of CGP 12177 in these mice.

Complementary results were obtained in β1-AR KO mice. Genetic ablation of the β1-AR eliminated the high-affinity, CGP 20712-sensitive activation of adenylyl cyclase by CGP 12177. Instead, brown fat membranes retained low-affinity, CGP 20712-insensitive activation by CGP 12177. The pharmacological properties of this receptor are consistent with those of recombinant β3-AR (Granneman et al., 1991). The fact that the effects of CGP 12177 that we attribute to β1-AR and β3-AR in wild-type mice are absent in the respective KO models provides compelling evidence that these subtypes mediate the complete actions of CGP 12177 in brown fat.

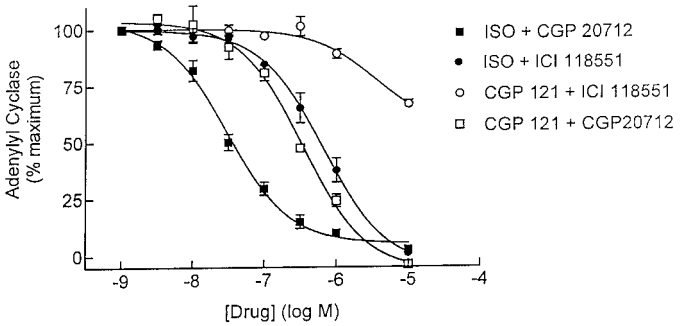

Fig. 5. Inhibition of isoproterenol- (ISO) and CGP 12177-stimulated adenylyl cyclase activity in brown fat membranes of β3-AR-KO mice by CGP 20712 and ICI 118,551. Membranes were stimulated with 50 nM agonists and indicated concentrations of antagonists. Values are means ± S.E. for three experiments.

A key conclusion of the present study is that CGP 12177 activates natively-expressed β1-AR and does not require overexpression, as supposed previously (Pak and Fishman, 1996). These data directly challenge the use of CGP 12177 to define responses mediated by β3-AR and the hypothetical β4-AR. For example, the existence of functional β3-AR on human white adipocytes has been inferred largely from studies of CGP 12177-induced lipolysis (Lönnqvist et al., 1993; Enocksson et al., 1995; Hoffstedt et al., 1996; Tavernier et al., 1996). However, β3-AR-selective phenethanolamine agonists, which are as effective as CGP 12177 in activating recombinant β3-AR, either fail to activate lipolysis in human fat cells or do so via interactions with β1/2-AR (Bousquet-Melou et al., 1995; Arch and Wilson, 1996, Tavernier et al., 1996; Umekawa et al., 1996; Galitzky et al., 1997; Sennitt et al., 1998). Additionally, CGP 12177 responsiveness in human fat cells correlates highly with isoproterenol-responsiveness, which seems to be mediated exclusively by β1/2-AR (Lönnqvist et al., 1993). In this regard, β1-AR mRNA is at least 100 times more abundant than β3-AR mRNA in human white fat (Granneman, 1995; Deng et al., 1996). Together, these data suggest that CGP 12177-induced lipolysis in humans is mediated mainly, if not exclusively, via β1-AR. Whether the weak lipolytic responses to novel aryloxpropanolamines (Sennitt et al., 1998) are mediated partly or exclusively via atypical interactions with β1/2-AR is not clear. Our data with the aryloxpropanolamine CGP 12177, however, indicates that resistance to standard β-AR blockade does not necessarily define interactions with β3-AR.

More recently, CGP 12177 has been used to propose the existence of a novel β-AR subtype, the β4-AR (Kaumann and Molenaar, 1996, 1997; Galitzky et al., 1997; Ito et al., 1998). However, the main features of this receptor, including lack of activation by β3-AR-selective phenethanolamines and resistance (relative to catecholamines) to blockade by various selective and nonselective β-AR antagonists, are consistent with those of β1-AR (Kaumann and Molenaar et al., 1997; Molenaar et al., 1997). Although the magnitude of resistance to β-AR blockers observed in the present study is somewhat less that that reported for the putative β4-AR, the present work is the first to compare native and recombinant receptors under identical conditions. More importantly, the present experiments with β1-AR and β3-AR KO mice indicate that actions of CGP 12177 in brown fat can be explained completely by its interactions with β1-AR and β3-AR. Although we have not explored whether myocardial β1-AR mediate chronotropic effects of CGP 12177, it is likely, given that this tissue expresses high levels of β1-AR and that the nonselective β-AR agonist isoproterenol fails to increase heart rate in β1-AR KO mice (Rohrer et al., 1996).

Antagonist affinity values (pA_2 or pK_B value) are widely used to define receptor subtypes, because classic receptor theory holds that the potency of an antagonist for a given receptor remains constant regardless of the agonist used to elicit the response (Kenakin, 1982, 1992). Indeed, the discrepancy between antagonist affinities for catecholamine- and CGP 12177-induced responses led to the proposal of the

β4-AR. Although a full analysis of CGP 12177 interactions with β1-AR is beyond the scope of the present study, it seems that this compound interacts with distinct sites/states of the β1-AR mediating activation and inhibition of activity (A. A. Konkar and J. G. Granneman, in preparation; see also Pak and Fishman, 1996). Current receptor theory models suggest that agonists, antagonists, and inverse agonists interact with (or stabilize) distinct states of the receptor (Gether and Kobilka, 1998). Within this context, activating concentrations of CGP 12177 seem to interact with a site/state of β1-AR that is poorly recognized by propranolol or isoproterenol. Thus, although the present results can be interpreted within the framework of classic receptor theory to indicate that the "receptor" sites/states for CGP 12177 and catecholamines activation are distinct, they demonstrate that these "receptor sites" need not exist on different proteins.

In summary, β1-AR mediate the β3-AR-independent actions of CGP 12177 in mouse brown fat. This activation occurs through a receptor site/state that is pharmacologically distinct from that activated by catecholamines. Receptor classification based on CGP 12177 agonist activity must clearly account for atypical interactions with β1-AR. Whether the unique interaction of aryloxypropanolamine agonists with β1-AR can be exploited for selective activation of cardiovascular and metabolic responses will be an important goal of future research.

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