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Isoproterenol and selective agonists stimulate similar atypical β-adrenoceptors in rat adipocytes

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Abstract—We have demonstrated previously that (−)isoproterenol triggers lipolysis in rat epididymal fat cells by stimulating both classical (β1, β2) and atypical β-adrenoceptors. The contribution of the classical β-adrenoceptors can be blocked by addition of 3 nM CGP12177{*di*-4-3[(1,1-dimethylethyl)amino]-(2-hydroxylpropoxy)1,3-dihydro-2*H*-benzimidazol-2-one hydrochloride}. At higher concentrations, CGP12177 triggers lipolysis also, but by stimulating atypical β-adrenoceptors only. To find out whether (−)isoproterenol and CGP12177 stimulate similar atypical β-adrenoceptors, we compared their interaction with recognised β3-adrenoceptor antagonists: CGP20712 {1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-propan-2-ol} (β1-selective), ICI118551 [erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol] (β2-selective) and the stereoisomers as well as the racemic mixture of propanolol (non-β1/β2-subtype selective) and of metoprolol (β1-selective). There was a highly significant relationship (r = 0.93) between the potencies of these antagonists for inhibiting the lipolytic response to (−)isoproterenol (in the absence of classical β-adrenoceptor stimulation) and CGP12177. In both cases, propranolol and metoprolol showed also the same degree of stereoselectivity. These findings suggest that (−)isoproterenol and CGP12177 stimulate the same type and/or form of atypical β-adrenoceptors in rat epididymal adipocytes.

The existence of "atypical" β-adrenoceptors in rat adipocytes has long been suspected due to the unusually low potency with which β-adrenergic antagonists inhibit lipolytic responses [1, 2]. Certain agonists, such as BRL37344,* are now also recognised to stimulate atypical β-adrenoceptors more potently than the "classical" (i.e. β1- and β2-) adrenoceptors [3]. CGP12177 is even more selective; it is a potent β1- and β2-adrenergic antagonist, but it is an agonist for atypical β-adrenoceptors [4–6]. Making use of this dual action of CGP12177, we recently evidenced the coexistence of classical and atypical β-adrenoceptors in rat epididymal adipocytes [6]. At low concentrations, CGP12177 inhibited the lipolytic response to (−)isoproterenol. Since CGP12177 is a potent antagonist (with sub-nanomolar affinity) for β1- and β2-adrenoceptors [7], we ascribed this inhibition to the blockade of classical β-adrenoceptors [6]. However, the inhibition was only partial (maximally 43%, at 3 nM CGP12177) and the remaining response was ascribed to the stimulation of atypical β-adrenoceptors by (−)isoproterenol. At higher concentrations, CGP12177 triggered lipolysis on its own (EC_{50} = 68 nM) to almost the same degree (94%) as the full agonist (−)isoproterenol. This confirmed the presence of atypical β-adrenoceptors.

CGP12177 has been shown to behave either as a partial [5] or as a full agonist [4, 6], and it is not clear whether this variability reflects differences in efficacy of the drug or receptor heterogeneity. Indeed, little is still known about the atypical β-adrenoceptors, and the possibility has been raised that they represent a large, hitherto hidden group of β-adrenoceptors possessing peculiar pharmacological properties rather than a single species [1, 8]. In addition to a putative diversity in primary amino acid sequence, atypical β-adrenoceptors might also adopt different functional and/or conformational states resulting from post-transcriptional modifications such as phosphorylation. Such functional and structural heterogeneity has been particularly well documented for β2-adrenoceptors [9]. In the present study, we evaluate these possibilities by comparing the pharmacological profiles of the CGP12177- and (−)isoproterenol-stimulated atypical β-adrenoceptors in rat adipocytes.

* Abbreviations: pA_2, negative logarithm of the molar concentration of an antagonist which reduces the effect of a dose of an agonist to that of half the dose; BRL37344, *di*-4-2′-{2-hydroxy-2-(3-choloro-phenyl)-ethylamino}propyl phenoxyacetic acid sodium salt sesquihydrate (*RR*, *SS* diastereoisomer); CGP12177, *di*-4-3[(1,1-dimethylethyl)amino]-(2-hydroxylpropoxy)1,3-dihydro-2*H*-benzimidazol-2-onehydrochloride; ICI118551, erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol; CGP20712, 1-[2-((3-carbamoyl-4-hydroxy)-phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-propan-2-ol.

Materials and Methods

Kind gifts: (−)-, (+)metoprolol HCl, (±)metoprolol tartrate from Astra-Hassle (Molndal, Sweden); (−)-, (+)propranolol HCl and ICI118551 from ICI Pharmaceuticals (Macclesfield, U.K.); CGP20712A and CGP12177 from Ciba-Geigy (Basle, Switzerland). All other materials were from commercial sources as stated in Ref. 6. Isolation of epididymal adipocytes from male Sprague–Dawley OFA rats (180–200 g) was done according to Rodbell [10] with minor modifications [6]. Washed adipocytes (3%, final lipocrit) were added to a mixture of the drugs of interest in modified Krebs–Ringer buffer (pH 7.4), containing 50 mg/L sodium meta-bisulfite, 10 μg/mL adenosine deaminase, 4% (w/v) bovine serum albumin (500 μL final volume), and incubated for 60 min at 37°. The tubes were then centrifugated (1600 *g*, 4°) and the glycerol concentration in the infranatant was determined by a bioluminometric kinetic assay [6].

Results and Discussion

To find out whether (−)isoproterenol and CGP12177 stimulate similar atypical β-adrenoceptors, we compared the inhibitory potencies of the following recognised [8] β3-adrenoceptor antagonists: CGP20712 (β1-selective), ICI118551 (β2-selective), and the enantiomers as well as the racemic mixture of propranolol (non-β1/β2-subtype selective) and of metoprolol (β1-selective). Control experiments revealed that these antagonists are unable to stimulate lipolysis on their own and that, at 1 mM, they

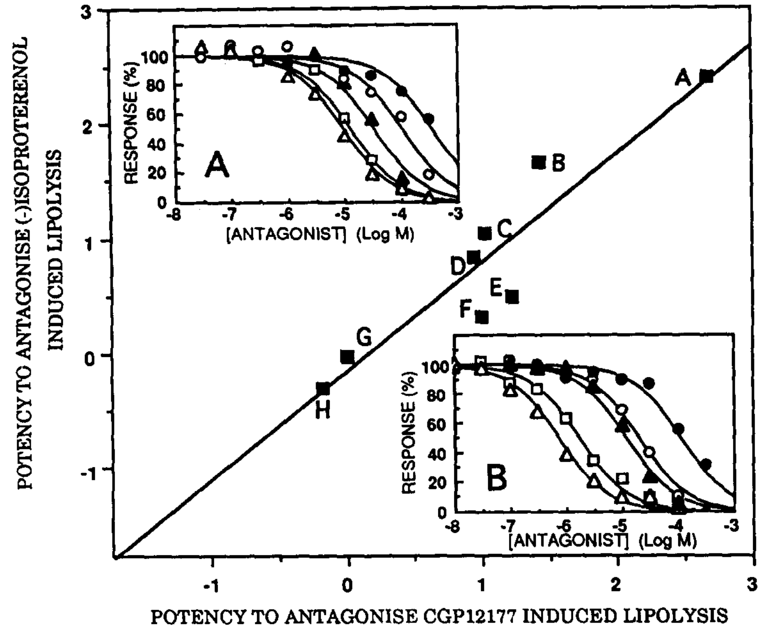

Fig. 1. Inhibition of the "atypical" β-adrenoceptor-mediated lipolytic response to CGP12177 and to (−)isoproterenol by β-adrenergic antagonists. Glycerol concentration (over basal level) was measured after incubation of rat adipocytes with 140 nM CGP12177 (Panel A) or 10 μM (−)isoproterenol (Panel B) (with 3 nM CGP12177 added to inhibit classical β-adrenoceptors), either in the absence of antagonist (100% response) or in the presence of increasing concentrations (abscissa) of ICI118551 (▲), CGP20712 (●) and (+)-, (±)- and (−)propranolol (○,□,△). Basal glycerol concentration (no agonist added) = 1–2.5 mg/L, 100% response = 15–25 mg/L. Main figure: relationship between antagonist potencies to inhibit CGP12177- (abscissa) and (−)isoproterenol- induced lipolysis (ordinate) (r=0.93). Antagonists are: (+), (±)- and (−)propranolol (C,G,H); (+)-, (±)- and (−)metoprolol (A,E,F); CGP20712 (B) and ICI118551 (D). Potencies are presented as Log(IC_{50}) and relative to (±)propranolol.

produce a parallel rightward shift of the dose–response curve of CGP12177 (data not shown). Since some of the antagonists only produced a small shift, their potencies were evaluated on the basis of dose–inhibition studies.

The atypical β-adrenoceptors were stimulated either by 10 nM (−)isoproterenol (in the presence of 3 nM CGP12177 to block β1- and β2-adrenoceptors) or by 140 nM CGP12177. These concentrations are about twice their EC_{50} [6]. Any tangible contribution of β1- and β2-adrenoceptors to the (−)isoproterenol-mediated lipolytic response under the present conditions can be excluded because of the very low potencies of CGP20712 and ICI118551 (Fig. 1A and B). The dose–inhibition curves are steep for all the antagonists (Hill coefficients close to unity). Their IC_{50} values, calculated according to a one-site model, are depicted in Table 1. These data reveal that, for both agonists, the receptors are stereoselective for propranolol and for metoprolol; i.e. the levorotatory isomer is about twice as potent as the racemic mixture and >20 (for propranolol) to >100 (for metoprolol) times more potent than the dextrorotary isomer. As shown in Fig. 1, there is a good correlation between the potencies [as Log(IC_{50}) values] of the antagonists for inhibiting (−)isoproterenol- and CGP12177-mediated lipolysis ($r = 0.93$). These data reveal that the atypical β-adrenoceptors which are stimulated by these two agonists are the same. We could therefore calculate the pA_2 values of the different antagonists (Table 1) from their $IC_{50}/IC_{50\ propranolol}$ ratio by the following equation: $pA_2 = -\mathrm{Log}\ (IC_{50}/IC_{50\ propranolol}) + pA_{2\ propranolol}$. The pA_2 of (±)propranolol for the atypical β-adrenoceptors ($pA_2 = 5.8$) has been determined previously from the rightward shifts that it imposes on CGP12177 dose–response curves [6].

As shown in Table 1, each antagonist displays similar pA_2 values for inhibiting the isoproterenol- and CGP12177-mediated response. pA_2 values range from 6 for the most potent antagonist, (−)propranolol, to 3 for (+)metoprolol. Such low antagonist affinities are characteristic of atypical β-adrenoceptors, and this property even formed the initial basis for the distinction between these receptors and their β1- and β2-counterparts [2]. This distinction is particularly striking for the β1-adrenoceptor-selective antagonist CGP20712. Its affinity for rat sinoatrial β1-adrenoceptors ($pA_2 = 9.44$) [11] is 100,000 times higher than for the atypical β-adrenoceptors in rat adipocytes: i.e. $pA_2 = 4.13$, 4.37 and 4.61 for inhibiting the lipolytic responses to (−)isoproterenol, CGP12177 (Table 1) and BRL37344, respectively [12]. The β2-selective antagonist ICI118551 possesses also a more than 1000-fold higher affinity for rat tracheal β2-adrenoceptors ($pA_2 = 8.72$) [3] than for the atypical β-adrenoceptors: i.e. $pA_2 = 4.95$, 4.85 and 5.33 (same agonists as used for CGP20712).

Atypical β-adrenoceptors are also known to be stereoselective, but their degree of stereoselectivity for antagonist molecules has been suggested to be less pronounced than for the other β-adrenoceptor subtypes [2, 12]. The present study complies with this opinion for propranolol. Its stereoselectivity index (i.e. $pA_{2(-)\,enantiomer} - pA_{2(+)\,enantiomer}$) for the atypical β-adrenoceptors (1.20 and 1.32 for inhibiting the CGP12177- and isoproterenol-mediated response, respectively) is well below the index of about 2 for the β1- and β2-adrenoceptors in rat atrium

Table 1. Antagonist potencies (IC_{50}) and affinity (pA_2) values for inhibiting CGP12177- and (−)isoproterenol-mediated lipolysis (via atypical β-adrenoceptors)

	Agonist: isoproterenol			Agonist: CGP12177		
Antagonists	IC_{50} (μM)	pA_2	nH	IC_{50} (μM)	pA_2	nH
ICI118551	12 ± 1	4.9	1.05	115 ± 10	4.9	1.18
CGP20712	79 ± 6	4.1	0.88	350 ± 37	4.4	0.93
(−)Propranolol	0.92 ± 0.13	6.1	0.91	8.7 ± 1.9	6.0	0.84
(±)Propranolol	1.7 ± 0.2	5.8	1.08	13 ± 3	5.8	1.06
(+)Propranolol	19 ± 1	4.8	0.94	140 ± 8	4.8	0.96
(−)Metoprolol	3.7 ± 0.9	5.5	0.98	130 ± 33	4.8	0.88
(±)Metoprolol	5.4 ± 0.7	5.3	1.04	220 ± 5	4.6	1.06
(+)Metoprolol	440 ± 120	3.4	0.94	6200 ± 960	3.1	0.95

Antagonist IC_{50} values were determined from inhibition data such as in Fig. 1A and B by computer-assisted curve fitting. All Hill coefficients are close to unity. Each value is given as the mean ± SE of three to eight experiments.

and diaphragm [2]. However, a different picture emerges for the β1-selective antagonist metoprolol. Its stereoselectivity index for the atypical β-adrenoceptors (between 1.68 and 2.07) is lower than for the β1-adrenoceptors in guinea pig left ventricles (index = 2.73) [13] but in the same range as for the β2-adrenoceptors in guinea pig soleus muscle (index = 1.76) [13]. This indicates that antagonists are not necessarily less stereoselective for atypical β-adrenoceptors than for the classical β-adrenoceptors.

The lipolysis experiments by Langin *et al.* [5] stipulate that CGP12177 is only a partial agonist; CGP12177 stimulated the glycerol release by no more than 36% of the level attained with (−)isoproterenol. In contrast, we have shown previously that CGP12177 stimulates the release of glycerol to almost the same level (94 ± 2%) as (−)isoproterenol [6]. CGP12177 also behaved as a full agonist in the study of Mohell and Dicker [4], where it stimulated oxygen consumption in brown adipocytes to the same degree as noradrenaline. The higher "intrinsic activity" of CGP12177 in these two latter studies may be imputed to a higher efficacy of the stimulus–response coupling. For partial agonists, such as CGP12177, an increase in efficacy should indeed raise maximal responsiveness and ultimately also produce a leftward shift of their dose–response curves (as compared to receptor–occupancy curves). In the same experimental system, such a shift is more pronounced for full agonists than for partial agonists. This implies: (i) that a full agonist needs to occupy less receptor sites than a partial agonist to produce the same degree of submaximal response and (ii) that antagonists display higher IC_{50} values for inhibiting the response evoked by a partial agonist (at a given concentration, L_p) than by an equieffective concentration (L_f) of a full agonist. This latter implication arises from the fact that the IC_{50} of the antagonist is related to its equilibrium dissociation constant (K_i) by the equation of Cheng and Prusoff (i.e. $IC_{50} = K_i \times (1 + L/K_D)$, with L = concentration and K_D = equilibrium dissociation constant of the agonist) and because $L_p/K_D > L_f/K_D$. The observation that all the antagonists inhibit the CGP12177-mediated lipolytic response with about a 10 times higher IC_{50} than the (−)isoproterenol-mediated response (Table 1) can thus be explained by the partial agonism of CGP12177.

Our results confirm that CGP12177 is a selective, partial agonist for the atypical β-adrenoceptors, which can be made to behave as a full agonist when the experimental conditions allow a high efficacy of the stimulus–response coupling in rat epididymal fat cells. The CGP12177- and (−)isoproterenol-stimulated atypical β-adrenoceptors display the same pharmacological profile for a series of antagonists. It can thus be concluded that both agonists stimulate the same type and/or form of atypical β-adrenoceptors.

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