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Constitutively active mutants of the β1-adrenergic receptor

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Abstract We provide the first evidence that point mutations can constitutively activate the β_1-adrenergic receptor (AR). Leucine 322 of the β_1-AR in the C-terminal portion of its third intracellular loop was replaced with seven amino acids (I, T, E, F, C, A and K) differing in their physico-chemical properties. The β_1-AR mutants expressed in HEK-293 cells displayed various levels of constitutive activity which could be partially inhibited by some beta-blockers. The results of this study might have interesting implications for future studies aiming at elucidating the activation process of the β_1-AR as well as the mechanism of action of beta-blockers.

Key words: G protein-coupled receptor; Adrenergic receptor; Constitutive activity; Inverse agonism

1. Introduction

The adrenergic receptors (AR) mediate the functional effects of epinephrine and norepinephrine by coupling to several of the major signalling pathways modulated by G proteins. The AR family includes nine different gene products: three β (β_1, β_2, β_3), three α_2 (α_{2A}, α_{2B}, α_{2C}) and three α_1 (α_{1a}, α_{1b}, α_{1d}) receptor subtypes. Like all G protein-coupled receptors (GPCR), the ARs display seven transmembrane α-helices which contribute to form the ligand binding pocket, whereas amino acid sequences of the intracellular (i) loops mediate the interaction of the receptor with the G protein as well as with other signalling and regulatory factors [1].

We have previously reported that mutations of alanine 293 in the C-terminal portion of the 3i loop of the α_{1b}-AR could dramatically increase the constitutive activity of the receptor [2]. A constitutively active mutant of the β_2-AR (β_2-CAM) was constructed by mutating four residues in the C-terminal portion of its third intracellular loop including leucine 272 which is homologous to alanine 293 in the α_{1b}-AR [3]. The constitutive activity of the α_{2A}-AR could be increased by different mutations of the single residue threonine 348 corresponding to alanine 293 in the α_{1b}-AR [4]. Activating mutations in the C-terminal portion of the 3i loop have also been described for several other GPCRs [5].

The availability of both constitutively active GPCR mutants and cell systems overexpressing wild type GPCR has presented a useful tool to identify inverse agonists (or negative antagonists) which exhibit the defining property of inhibiting the agonist-independent activity of the receptors [6]. In particular, it has been previously demonstrated that several classical beta-blockers can behave either as partial agonists, neutral antagonists or inverse agonists in cells expressing the wild type β_2-AR or at the β_2-CAM [7,8].

The main aim of this study was to investigate whether mutations in the C-terminal portion of the third intracellular loop could activate the β_1-AR for which constitutively active mutants have not been described so far. To achieve this goal, leucine 322 of the β_1-AR (homologous to alanine 293 of the α_{1b}-AR, threonine 368 of the α_{2A}-AR and leucine 272 of the β_2-AR) was replaced with seven amino acids differing in their physico-chemical properties and the receptor mutants were tested for their ability to mediate agonist-independent accumulation of cAMP. Our study provides the first evidence that the β_1-AR can be activated by point mutations and might contribute to further elucidate the pharmacological effects of drugs acting at different β-AR subtypes.

2. Materials and methods

2.1. Drugs

Ligands were purchased from TOCRIS ((+) or (−)propranolol, timolol, betaxolol, ICI118,551, practolol, sotalol), Sigma (alprenolol, (±)propranolol, labetolol, metoprolol), RBI (CGP20712A) and Aldrich (atenolol). Carvedilol was a gift from Pfizer.

2.2. Mutagenesis and cell transfection

The cDNA encoding the human β_1-AR [9] was mutated by PCR-mediated mutagenesis technique using *Taq* DNA polymerase (Roche). The mutated DNA fragments obtained were digested with the appropriate enzymes and cloned into the expression vector pRK-5 containing the wild type β_1-AR cDNA. The cDNAs encoding the wild type human β_2-AR or the constitutively active β_2-CAM [3] were also in the expression vector pRK-5. HEK-293 cells, grown in DMEM Nut Mix F-12 (Gibco BRL) supplemented with 10% FCS and gentamicin (100 μg/ml), were transiently transfected with different cDNAs using the FuGENE 6 transfection reagent (Boehringer Mannheim) and harvested 48 h after the transfection. The transfected DNA was 0.5–1 μg/10^6 cells.

2.3. Ligand binding

Crude membranes were prepared from cells expressing the β-ARs and their mutants. The binding was performed at 25°C in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl and 5 mM EDTA. For saturation binding experiments of [^{125}I]CYP (DuPont-NEN, Boston, MA), the radioligand concentration ranged from 12 to 400 pM and 10^{-6} M alprenolol was used to determine non-specific binding. In competition binding experiments, the final concentrations of [^{125}I]CYP was 80 pM. Results of ligand binding experiments were analyzed using Prism 2.0 (GraphPAD, Software, San Diego, CA, USA).

2.4. cAMP measurements

Cells were transfected in 6-well dishes and incubated overnight in culture medium containing 2.5 μCi/ml [^{3}H]adenine (Amersham) with 5% fetal calf serum (FCS). After removal of the radioactive medium, the cells were incubated in culture medium without serum containing 10 mM HEPES and 1 mM isobutylmethylxanthine in the presence or absence of different ligands for 30 min. The medium was then removed and the cells were lysed in 1 ml of 2.5% perchloric acid. The [^{3}H]cAMP content of the cell lysate was determined as previously described by chromatography on Dowex/alumina columns [4].

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2.5. Statistical analysis

Statistical analysis was performed as indicated in the figure legends using Prism 2.0 (GraphPAD, Software, San Diego, CA, USA).

3. Results

3.1. Activating mutations of the β1-AR

Leucine 322 in the C-terminal portion of the 3i loop of the β_1-AR was mutated into seven amino acids differing in their physico-chemical properties. The wild type β_1-AR and its mutants (L322I, L322T, L322E, L322F, L322C, L322A and L322K) were expressed in HEK-293. The properties of the β_1-ARs were compared with those of the wild type β_2-AR and of its constitutively active mutant β_2-CAM [3] carrying four mutations in the C-terminal portion of the 3i loop of the receptor including the mutation of leucine 272 homologous to leucine 322 of the β_1-AR.

Cells expressing the various receptors did not display significant differences in the total incorporation of [^{3}H]adenine which was about 0.05%. On the other hand, clear differences were observed in the basal levels of cAMP in cells expressing the various receptors (Fig. 1). In cells expressing the wild type β_1-AR or β_2-AR the basal levels of cAMP were 90% higher than in control cells expressing the vector alone (cAMP was 166 ± 20 cpm/well for the vector alone, 316 ± 35* cpm/well for β_1-AR and 384 ± 38* for β_2-AR, mean ± S.E.M. of six experiments, $*P < 0.05$ compared to vector alone). Thus both the wild type β_1-AR and β_2-AR display a very small, but significant spontaneous activity.

In agreement with previous findings in COS-7 cells [3], expression of the β_2-CAM in HEK-293 cells resulted in a significant 2.7-fold increase of the basal cAMP accumulation as compared to cells expressing the wild type β_2-AR (Fig. 1). For L322I, L322T and L322E the agonist-independent activity was not significantly different from that of the wild type β1-

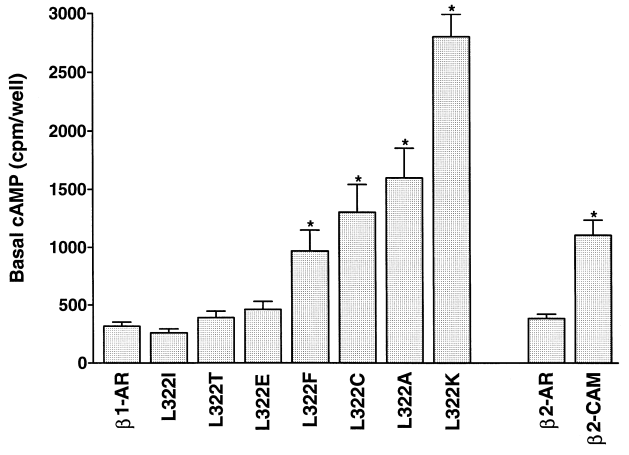

Fig. 1. Basal cAMP response in cells expressing the wild type and mutated β_1- and β_2-AR subtypes. [^{3}H]cAMP accumulation was measured in HEK-293 cells expressing the wild type or mutated receptors as described in Section 2. The β1-AR mutants carried mutations of leucine 322. The β_2-CAM carried four mutations in the 3i loop of the receptor as previously described [3]. Receptor expression ranged between 2.5 and 3 pmol/mg of protein for the β_1-ARs and 1 and 2 pmol/mg of protein for the β_2-ARs. Results are the mean ± S.E.M. of seven independent experiments. Statistical significance was analyzed by unpaired Student's *t*-test, $*P < 0.05$. Basal of the mutants was compared to that of their respective wild type receptor.

Table 1
Effect of ligands on the cAMP levels in cells expressing the wild type or mutated β-ARs

	β1-ARs							β2-ARs	
	β1-AR	L322I	L322T	L322E	L322F	L322C	L322A	L322K	β2-AR	CAM
Basal	316	259	390	461	967	1 303	1 599	2 826	373	1 014
Isoproterenol (10^{-4})	15 228 *(48.2)*	19 617 *(75.7)*	17 875 *(45.8)*	18 760 *(40.7)*	23 992 *(24.8)*	25 242 *(19.4)*	17 314 *(10.8)*	18 477 *(6.5)*	14 949 *(40.1)*	15 549 *(15.3)*
(−)Epinephrine (10^{-4})	9 887 *(31.3)*	–	–	–	–	–	–	11 553 *(4.1)*	8 861 *(23.8)*	9 277 *(9.2)*
(−)Alprenolol (10^{-6})	2 586 *(8.2)*	3 533 *(13.6)*	4 192 *(10.8)*	4 425 *(9.6)*	4 910 *(5.1)*	4 643 *(3.6)*	5 688 *(3.6)*	5 835 *(2.1)*	2 523 *(6.8)*	4 517 *(4.5)*
Carvedilol (10^{-5})	1 162 *(3.7)*	–	–	–	–	–	–	6 222 *(2.2)*	799 *(2.1)*	2 851 *(2.8)*
Labetalol (10^{-6})	1 909 *(6.0)*	2 172 *(8.4)*	2 854 *(7.3)*	3 178 *(6.9)*	3 557 *(3.7)*	3 922 *(3.0)*	4 647 *(2.9)*	5 352 *(1.9)*	3 844 *(10.3)*	9 974 *(9.8)*
(+)Propranolol (10^{-5})	654 *(2.1)*	514 *(2.0)*	940 *(2.4)*	896 *(1.9)*	2 459 *(2.5)*	2 820 *(2.2)*	4 262 *(2.7)*	5 505 *(2.0)*	975 *(2.6)*	2 838 *(2.8)*
(−)Propranolol (10^{-6})	1 697 *(5.4)*	1 463 *(5.7)*	2 451 *(6.3)*	1 827 *(4.0)*	4 313 *(4.5)*	4 894 *(3.8)*	4 884 *(3.1)*	4 979 *(1.8)*	943 *(2.5)*	2 440 *(2.4)*
(±)Propranolol (10^{-6})	1 954 *(6.2)*	1 400 *(5.4)*	2 446 *(6.3)*	1 955 *(4.2)*	3 613 *(3.7)*	3 904 *(3.0)*	5 326 *(3.3)*	5 064 *(1.8)*	568 *(1.5)*	1 568 *(1.6)*
Sotalol (10^{-5})	238 *(0.8)*	262 *(1.0)*	387 *(1.0)*	397 *(0.9)*	867 *(0.9)*	1 090 *(0.8)*	1 763 *(1.1)*	2 816 *(1.0)*	560 *(1.5)*	1 891 *(1.9)*
(−)Timolol (10^{-6})	1 209 *(3.8)*	1 022 *(4.0)*	2 263 *(5.8)*	1 405 *(3.1)*	3 956 *(4.1)*	3 385 *(2.6)*	4 969 *(3.1)*	4 952 *(1.8)*	399 *(1.1)*	1 552 *(1.1)*
Atenolol (10^{-5})	447 *(1.4)*	285 *(1.1)*	336 *(0.9)*	379 *(0.8)*	888 *(0.9)*	989 *(0.8)*	1 196 *(0.8)*	2 924 *(1.0)*	399 *(1.1)*	1 112 *(1.1)*
Betaxolol (10^{-5})	318 *(1.0)*	302 *(1.2)*	273 *(0.7)*	320 *(0.7)*	530 *(0.6)*	643 *(0.5)*	807 *(0.5)*	2 232 *(0.8)*	473 *(1.3)*	910 *(0.9)*
CGP20712A (10^{-5})	338 *(1.1)*	316 *(1.2)*	350 *(0.9)*	228 *(0.5)*	435 *(0.5)*	329 *(0.3)*	742 *(0.5)*	1 878 *(0.7)*	828 *(2.2)*	1 834 *(1.8)*
(±)Metoprolol (10^{-5})	334 *(1.1)*	257 *(1.0)*	385 *(1.0)*	362 *(0.8)*	351 *(0.4)*	1 118 *(0.9)*	910 *(0.6)*	2 521 *(0.9)*	424 *(1.1)*	953 *(0.9)*
Practolol (10^{-5})	2 903 *(9.2)*	3 236 *(12.5)*	3 617 *(9.3)*	3 836 *(8.3)*	6 451 *(6.7)*	6 066 *(4.7)*	7 404 *(4.6)*	8 596 *(3.0)*	2 353 *(6.3)*	4 840 *(4.8)*
ICI118,551 (10^{-5})	258 *(0.8)*	228 *(0.9)*	326 *(0.8)*	326 *(0.7)*	466 *(0.5)*	830 *(0.6)*	914 *(0.6)*	2 157 *(0.8)*	253 *(0.7)*	566 *(0.6)*

[^{3}H]cAMP levels are expressed as cpm/well. Basal indicates the cAMP levels of cells expressing the receptors in the absence of ligands. Numbers in parentheses indicate the fold of basal. Ligands have been used at different maximal concentrations as indicated (M). Results are the mean of two experiments which did not differ by more than 40%.

AR. In contrast, for the other mutants the basal activity was 3–9-fold greater (L322F < L322C < L322A < L322K) than that of the wild type β1-AR (Fig. 1). The L322A and L322K mutants displayed a constitutive activity much greater than that observed for β_2-CAM.

These results demonstrate that mutations of leucine 322 can constitutively activate β1-AR supporting the notion that the C-terminal portion of the 3i loop is an important switch regulating the activation of many GPCRs [1].

3.2. Effect of different ligands on receptor-mediated cAMP accumulation

As shown in Table 1, stimulation of the wild type β_1-AR and β_2-AR with isoproterenol or epinephrine resulted in a large increase of cAMP accumulation which was similar for the two receptors. Stimulation of cells with agonists could also increase the cAMP response mediated by β_2-CAM and by the β_1-AR mutants. The maximal cAMP levels induced by isoproterenol for the wild type β-ARs and their mutants ranged between 15 000 to 25 000 cpm/well. However, the cAMP increase expressed as fold of basal was smaller for the L322F, L322C, L322A, L322K and β_2-CAM as compared to the wild type receptors because of their increased constitutive activity (Table 1).

Fig. 2 shows the effect of different beta-blockers on the response mediated by the wild type β_1-AR and β_2-AR or by their respective most active mutants, L322K and β_2-CAM. Several beta-blockers displayed partial agonist activity at both the wild type receptors and their constitutively active mutants. However, their partial agonism was very small as compared to the effect of isoproterenol, as shown in Table 1. The non-selective blockers alprenolol, carvedilol, labetalol, (+) and (−)propranolol and the β_1-selective practolol behaved as weak partial agonists at both the β_1 and β_2-ARs (Fig. 2). Timolol displayed weak partial agonism at the β_1-ARs and neutral antagonism at the β_2-ARs. Vice versa, sotalol was a very weak partial agonist at the the β_2-ARs, being neutral at the β_1-ARs.

A set of three beta-blockers, i.e. the β_1-selective betaxolol and CGP20712A and the β_2-selective ICI118,551, displayed some differences at the constitutively active L322K and β_2-CAM. All three compounds were partial inverse agonists at the L322K. In contrast, the basal activity of β_2-CAM could be partially inhibited only by betaxolol and ICI118,551, CGP20712A being neutral (Fig. 2). Inverse agonism at the wild type receptors was difficult to assess because of their low spontaneous activity.

The effects of various ligands observed at the L322K mutant were conserved at the other receptor mutants carrying different mutations of L322 (Table 1). In particular, betaxolol, CGP20712A, and ICI118,551 could also partially inhibit the basal activity of the other constitutively active mutants L322F, L322C and L322A.

3.3. Ligand binding affinity for the wild type β_1-AR and mutated receptors

The allosteric ternary complex model of receptor activation [3] predicts that constitutively active receptor mutants display increased affinity for agonists and decreased affinity for inverse agonists as compared to wild type GPCRs whereas the affinity of neutral antagonists should be similar [8]. To test this hypothesis, the affinity of the agonists, isoproterenol and epinephrine, as well as of most beta-blockers was measured in membranes derived from HEK-293 cells expressing the wild type β_1-AR or its mutants (Table 2).

In agreement with the predictions of the allosteric ternary complex model [3], the affinity of isoproterenol and epinephrine was increased at the receptor mutants in a manner which was, at large extent, related to their constitutive activity. In fact the K_i values of isoproterenol and epinephrine at the most active mutant, L322K, were 200- and 100-fold smaller, respectively, as compared to those at the wild type β_1-AR (Table 2). The L322F, L322C and L322A mutants characterized by an intermediate level of constitutive activity displayed a 10-fold increase of their affinities for both isoproterenol and epinephrine. The L322E mutant was also characterized by a 10-fold increase of affinity for both agonists despite the fact that the small increase of its constitutive activity as compared to that of the wild type receptor was not significant. As previously reported, the affinity of isoproterenol for β_2-CAM was 25-fold greater than that for the wild type β_2-AR (results not shown).

In contrast, the K_i values of all the beta-blockers tested

Table 2
Ligand binding affinity for the wild type β1-AR and its mutants carrying mutations of L322

Ligand	β1-AR	L322I	L322T	L322E	L322F	L322C	L322A	L322K
Isoproterenol	6.55	6.33	6.96	7.43	7.39	7.23	7.52	8.46
(−)Epinephrine	4.84	4.68	5.44	5.84	5.61	5.55	5.9	7.15
(−)Alprenolol	8.17	8.21	8.18	8.41	8.24	8.18	8.29	8.31
Labetalol	7.13	7.12	7.26	7.76	7.24	7.19	7.32	7.08
(+)Propranolol	6.36	6.29	6.33	6.51	6.30	6.25	6.35	6.40
(−)Propranolol	8.47	8.52	8.67	8.81	8.84	8.72	8.65	8.78
(±)Propranolol	8.22	8.47	8.58	8.48	8.46	8.40	8.35	8.49
Sotalol	5.28	5.39	5.72	5.53	5.66	5.57	5.65	5.54
(−)Timolol	8.19	8.33	8.29	8.29	8.22	8.12	8.67	8.91
Atenolol	6.26	6.43	6.52	6.69	6.77	6.78	6.59	6.58
Betaxolol	7.80	7.66	8.08	8.08	8.07	7.88	8.07	8.06
CGP20712A	7.69	7.86	8.05	8.02	8.06	7.93	8.12	8.04
(±)Metoprolol	6.74	6.69	7.05	7.17	7.04	6.98	7.16	7.18
Practolol	5.77	5.58	5.94	5.91	5.89	5.86	6.00	6.16
ICI118,551	6.15	5.99	6.15	6.06	6.09	6.05	6.25	6.19

The affinities expressed as pK_i were determined in competition binding experiments using [^{125}I]CYP in cell membranes as described in Section 2. The best fit of the competition curves was monophasic and the Hill coefficient ranged between 0.7 and 1. The results for the mutants are the mean of two experiments which did not differ by more than 40%. The results for the wild type receptor are the mean of three or four experiments.

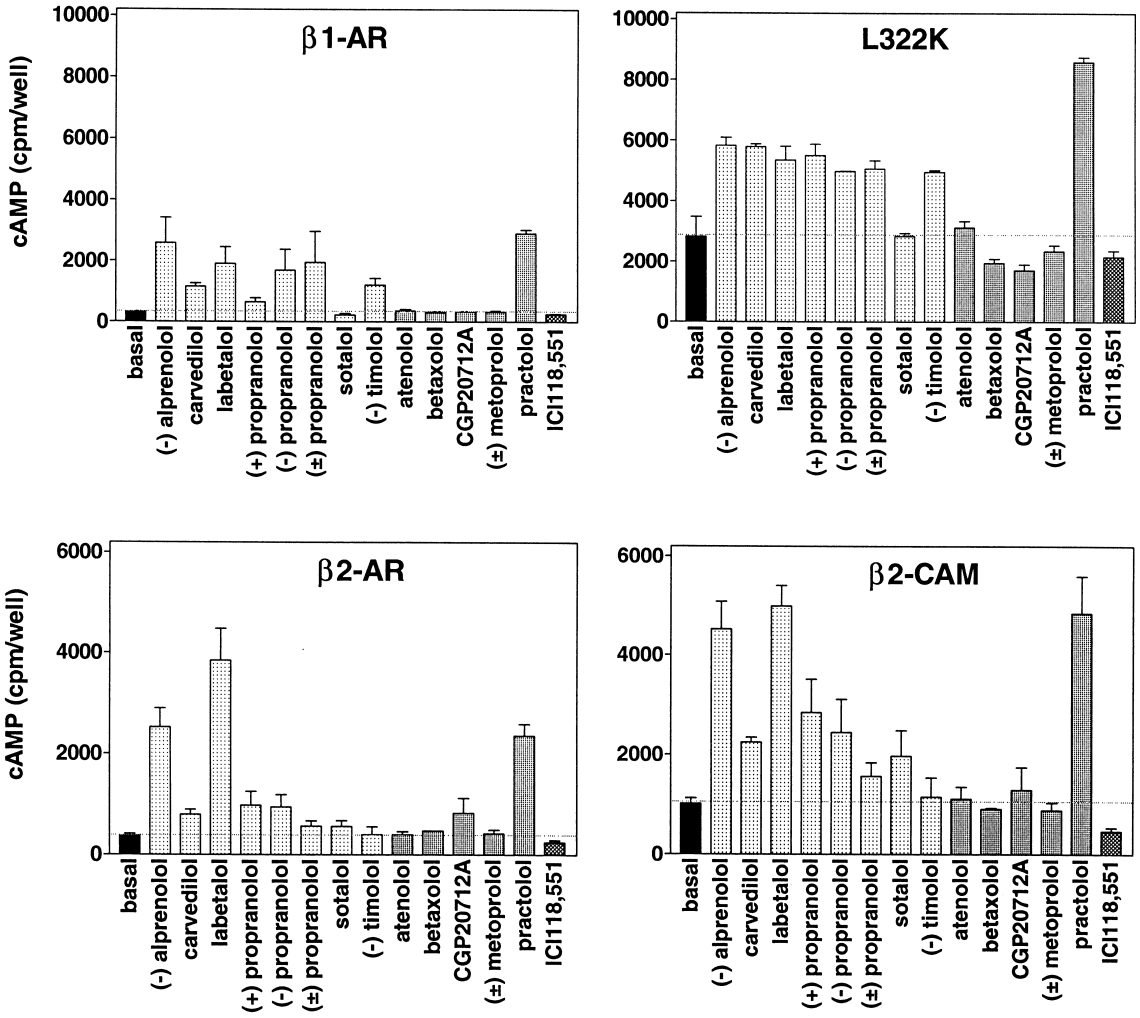

Fig. 2. Effects of different beta-blockers on the basal cAMP response mediated by the β_1- and β_2-AR subtypes and their constitutively active mutants. [^{3}H]cAMP accumulation was measured in HEK-293 cells expressing the wild type or mutated receptors as described in Section 2 in the absence (basal) or presence of different ligands. The final concentration of the ligands was as indicated in Table 1. The ligands are grouped according to their selectivity: non-selective (light stippling), β_1-selective (medium stippling) and β_2-selective (dark stippling). Results are the mean ± S.E.M. of three independent experiments. Statistical significance was analyzed by unpaired Student's *t*-test on the inhibition of basal: $P < 0.05$ (ligand versus basal) for ICI118,551 at L322K, wild type β_2-AR and β_2-CAM; for betaxolol at L322K and β_2-CAM; for CGP20712A at L322K.

were not different at the mutants as compared to the wild type β_1-AR (Table 2). Similarly, the K_d of [^{125}I]CYP was about 80 pM for all the receptors (results not shown). These results are not surprising and can find their interpretation in the framework of the allosteric ternary complex model [8]. As predicted by the relationship between the difference in ligand affinity between the CAM versus the wild type receptor and the isomerization constant *J* of the receptor, the change of affinity induced by an activating mutation can be much larger for full agonists than for inverse agonists depending on the value of *J* [8]. For example, the affinity of isoproterenol for β_2-CAM was 25-fold higher, whereas that of the full inverse agonist ICI118,551 was only 2-fold lower than for the wild type receptor.

4. Discussion

The main finding of our study is that point mutations can constitutively activate the β_1-AR for which activating mutations had not been described before. Several studies have focused so far on the β_2-AR whereas much less is known about the structure-relationship activity of the β_1-AR. It has been reported that the efficacy of coupling to Gs of the β_1-AR is lower than that of the β_2-AR in Ltk$^-$ mouse fibroblasts [10]. This was mainly demonstrated by the fact that the β_1-AR-mediated cAMP response was smaller than that elicited by equivalent densities of β_2-AR. In our study, the maximal agonist-induced response was similar for the two receptors, in agreement with the results obtained in another study comparing the receptors in CHW mouse fibroblasts [11]. In addition, the constitutive activity induced by some mutations of L322 was higher than that of the β_2-CAM. Altogether, these results demonstrate that in HEK-293 cells the β_1-AR can be at least as active as the β_2-AR.

Our findings on the effect of beta-blockers on the constitutive activity of the β_2-CAM are in agreement with those from a previous study demonstrating that among the ligands tested only betaxolol and ICI118,551 could inhibit the agonist-independent activity of β_2-CAM measured in membranes derived from CHO cells permanently expressing the receptor [3]. In

transgenic mice overexpressing the wild type β_2-AR, ICI118,551 also behaved as an inverse agonist inhibiting the agonist-independent myocardial activity [12]. However, the results of other studies investigating inverse agonism at the β_2-AR or its CAM are apparently divergent. In NG108-15 cells overexpressing β_2-CAM [13] not only betaxolol and ICI118,551 but also timolol and sotalol (which are very weak partial agonists in our study) behaved as inverse agonists on the receptor-mediated activation of adenylyl cyclase in membranes. In the study by Chidiac et al. in membranes derived from Sf9 cells overexpressing wild type β_2-CAM [7], most ligands tested displayed different degrees of inverse agonism according the following rank order timolol > propranolol > alprenolol > pindolol > labetalol > dichloroisoproterenol. Betaxolol and ICI118,551 were not tested in this study. Interestingly, this study reported that the degree of negative efficacy of propranolol was positively correlated with receptor expression. In addition, whereas in membranes most ligands behaved as partial inverse agonists, in whole cells most of them were weak partial agonists in agreement with our present results. In another interesting study it was reported that receptor desensitization as well as the initial state of the receptor can modulate the degree of negative efficacy of beta-blockers at the β_2-AR expressed in Sf9 cells [14].

On the basis of all these findings one might conclude that those beta-blockers with marked negative efficacy, i.e. betaxolol and ICI118,551, unequivocally display inverse agonism at the β_2-AR or its CAM under different experimental conditions. In contrast, other beta-blockers can display modest inverse agonism or partial agonism depending on the experimental conditions under which they have been tested, e.g. cell system expressing the receptor, levels of receptor expression, measurement of cAMP production in membranes versus whole cells, desensitization state of the receptor system.

Inverse agonism at the recombinant β_1-AR has not been reported before. However, the effect of some beta-blockers has been investigated in a physiological system on the response mediated by the β_1-AR in isolated guinea pig and human cardiomyocytes [15]. Interestingly, in this cell system the agonist-independent activation of calcium currents induced by the β_1-AR could be partially inhibited by atenolol and propranolol. ICI118,551 did not have any effect in the guinea pig cardiomyocytes, but it was not tested on the human cells. Inverse agonism was more pronounced in the presence of forskolin which sensitizes the cAMP-dependent calcium response. These findings differ from those of our present study in which atenolol and propranolol display some partial agonism at the recombinant human β_1-AR and its CAMs. As discussed before, the effect of beta-blockers on the spontaneous activity of either the β_1- or β_2-AR subtypes might depend to a large extent on the experimental system investigated.

In conclusion, the experimental results available so far do not support a general classification of beta-blockers based on their pharmacological properties at the β_1- or β_2-AR subtypes, i.e. neutral antagonism, inverse agonism and partial agonism. As a consequence, the correlation between the in vivo effects of beta-blockers and their pharmacological properties as well as the clinical significance of inverse agonism remain to be further explored. In this respect, the constitutively active β_1-AR mutants described in this work might represent a new useful tool to further investigate, on one hand, the activation process of the β_1-AR and, on the other, the mechanism of action of beta-blockers.

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References

[1] Wess, J. (1997) FASEB J. 5, 346–354.
[2] Kjelsberg, M.A., Cotecchia, S., Ostrowski, J., Caron, M.G. and Lefkowitz, R.J. (1992) J. Biol. Chem. 267, 1430–1433.
[3] Samama, P., Cotecchia, S., Costa, T. and Lefkowitz, R.J. (1993) J. Biol. Chem. 268, 4625–4636.
[4] Ren, Q., Kurose, H., Lefkowitz, R.J. and Cotecchia, S. (1993) J. Biol. Chem. 268, 16483–16487. [Published erratum in J. Biol. Chem. 269 (1994) 1566].
[5] Scheer, A. and Cotecchia, S. (1997) J. Recept. Signal Transduct. Res. 17, 57–73.
[6] Milligan, G., Bond, R.A. and Lee, M. (1995) Trends Pharmacol. Sci. 16, 10–13.
[7] Chidiac, P., Hebert, T.E., Valiquette, M., Dennis, M. and Bouvier, M. (1994) Mol. Pharmacol. 45, 490–499.
[8] Samama, P., Pei, G., Costa, T., Cotecchia, S. and Lefkowitz, R.J. (1994) Mol. Pharmacol. 45, 390–394.
[9] Frielle, T., Collins, S., Daniel, K.W., Caron, M.G., Lefkowitz, R.J. and Kobilka, B.K. (1987) Pro. Natl. Acad. Sci. USA 84, 7920–7924.
[10] Levy, F.O., Zhu, X., Kaumann, A.J. and Birnbaumer, L. (1993) Proc. Natl. Acad. Sci. USA 90, 10798–10802.
[11] Green, S.A., Holt, B.D. and Liggett, S.B. (1992) Mol. Pharmacol. 41, 889–893.
[12] Bond, R.A., Leff, P., Johnson, T.D., Milano, C.A., Rockman, H.A., McMinn, T.R., Apparsundaram, S., Hyek, M.F., Kenakin, T.P., Allen, L.F. and Lefkowitz, R.J. (1995) Nature 374, 272–276.
[13] MacEwan, D.J. and Milligan, G. (1996) Mol. Pharmacol. 50, 1479–1486.
[14] Chidiac, P., Nouet, S. and Bouvier, M. (1996) Mol. Pharmacol. 50, 662–669.
[15] Mewes, T., Dutz, S., Ravens, U. and Jakobs, K.H. (1993) Circulation 88, 2916–2922.