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The effect of the thermostabilising mutations (M23 changes) and intracellular loop amino acid deletions (B36 changes) on whole cell binding to the turkey β-adrenoceptor expressed in CHO-K1 cells

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The turkey β-adrenoceptor (AR) has recently been determined to 2.7 Å resolution (Warne et al., 2008). However, in order for this to happen, several changes were made to the receptor: 30 amino acids were deleted from the N-terminus and 115 from the C-terminus (tβtrunc which became the starting point for the other mutations): the thermostability of the molecule was then increased by the introduction of 6 point mutations (R67S, M90V, Y227A, A282L, F327A, F338M; tβtruncM23); 30 amino acids were then deleted from the third intracellular loop (tβtruncB36) generating the final product tβtruncB36M23 (Warne et al., 2008). This study examined the binding characteristics of these mutant receptors.

CHO-K1 cells were transfected with lipofectamine, OPTIMEM and 10μg DNA per T75 flask. After 24hrs, the transfection reagents were removed and the cells left to recover in media for 24hrs. Cells were then plated into 96-well plates and grown to confluence over 24hrs. ^{3}H-CGP 12177 whole cell saturation and competition binding experiments were then performed as previously described (Baker 2005).

As can be seen from the Table, the affinity of ligands was reduced by the introduction of both the M23 and B36 mutations, however the final product, tβtruncB36M23, was still able to bind ligands and some with high affinity.

	tβtrunc			tβtruncM23			tβtruncB36			tβtruncB36M23	
CGP 20712A	-7.09 ± 0.05	21		-6.37 ± 0.10	8		-6.93 ± 0.04	8		-5.57 ± 0.05	8
Xamoterol	-6.33 ± 0.03	6		-5.46 ± 0.04	8		-5.94 ± 0.04	8		-4.74 ± 0.02	8
Bisoprolol	-6.36 ± 0.06	6		-5.58 ± 0.03	8		-6.02 ± 0.04	7		-4.92 ± 0.02	7
Betaxolol	-6.55 ± 0.02	5		-6.19 ± 0.02	8		-6.12 ± 0.05	7		-5.23 ± 0.03	7
Metoprolol	-6.23 ± 0.09	5		-5.51 ± 0.03	8		-5.74 ± 0.08	8		-4.75 ± 0.07	8
Propranolol	-8.24 ± 0.03	7		-7.63 ± 0.04*	5		-7.90 ± 0.05	7		-7.25 ± 0.03	7
Timolol	-8.53 ± 0.02	7		-7.70 ± 0.03*	5		-8.27 ± 0.03	8		-7.26 ± 0.03	8
ICI 118551	-6.75 ± 0.03	22		-6.67 ± 0.03	8		-6.60 ± 0.05	8		-5.87 ± 0.04	8
Cimaterol	-6.50 ± 0.05	7		-5.25 ± 0.06*	3		-6.17 ± 0.03	8		-4.76 ± 0.05	8
Clenbuterol	-6.60 ± 0.03	5		-5.64 ± 0.01	8		-6.37 ± 0.02	7		-5.19 ± 0.03	7
Formoterol	-6.41 ± 0.04	20		-5.25 ± 0.03	8		-6.11 ± 0.3	8		-4.62 ± 0.05	8
Salmeterol	-5.50 ± 0.02	22		-5.07 ± 0.11*	3		-5.41 ± 0.05	8		-4.82 ± 0.04	8

Table. Log K_D values as determined from ^{3}H-CGP 12177 whole cell binding. Values are mean ± s.e.m. determined from n separate experiments, each from a separate population of transiently transfected cells. * log K_D value determined from a stable cell line expressing the tβtruncM23 receptor

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Baker JG (2005) Br. J. Pharmacol. 144: 317-322

Warne T et al., (2008) Nature 454: 486-491