AGONIST-DEPENDENT CHANGES IN ANTAGONIST AFFINITY FOR THE HUMAN β3 -ADRENOCEPTOR

Jillian G Baker. Institute of Cell Signalling, University Of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH. UK.

The affinity of an antagonist for a given receptor is generally considered to be a fixed property of that specific receptor-ligand interaction. However studies of the human β2-adrenoceptor (AR) have shown a 10-fold difference in antagonist affinity which depends on the competing agonist used (Baker et al., 2003a). Antagonist affinity measurements at the human β1-AR also vary depending on the competing agonist and this has been attributed to the presence of at least two active sites of the receptor (Konkar et al., 2000; Baker et al., 2003b). The aim of this study was to determine whether the antagonist affinity measurements at the human β3-AR change with the nature of the competing agonist used.

CHO cells stably expressing the human β3-AR and a cAMP response element (CRE)-luciferase reporter gene were used Measurements of ^{3}H-cAMP accumulation and CRE-luciferase production were made as previously described (Baker et al., 2003b). In order to determine antagonist affinity measurements an antagonist with no intrinsic efficacy of its own was sought. Acebutolol, ICI 89406, nadolol, ephedrine, dopamine, propranolol, xamoterol, procaterol and timolol all stimulated an increase in CRE-luciferase and cAMP production at high concentrations. Atenolol, betaxolol, bisoprolol, bupranolol, CGP20712A, ICI 118551, metoprolol and sotalol were found to have no intrinsic efficacy. Several other ligands generally classed as β-antagonists had agonist activity (see Table). All agonist responses were inhibited by ICI 118551, however, the K_D values obtained for ICI 118551 were not constant and varied up to ten-fold depending on the competing agonist used. This was seen in both the short term cAMP assay and the longer term gene transcription assay and was not related to ligand efficacy (see Table). The cause of the range of antagonist affinities values obtained is therefore not the same as for that seen at the β2-AR and may be more akin to that seen at the β1-AR.

	^{3}H-cAMP accumulation				CRE-luciferase production			
agonist	Log EC_{50}	%isop	Log K_D ICI 118551	n	Log EC_{50}	%isop	Log K_D ICI 118551	n
terbutaline	-5.53±0.06	97.5±0.4	-6.24±0.09	5	-5.68±0.13	110.6±3.0	-6.13±0.05**	4
salbutamol	-5.76±0.08	88.8±3.0	-6.30±0.03	3	-5.76±0.12	108.2±2.0	-6.13±0.06**	4
cimaterol	-6.73±0.08	86.2±2.5	-6.33±0.07*	5	-6.71±0.04	106.3±1.8	-6.09±0.06**	5
BRL 37344	-7.38±0.05	79.2±3.4	-6.13±0.04	3	-7.51±0.03	99.2±1.9	-6.02±0.03**	17
ICI 215001	-7.06±0.06	60.3±2.9	-6.04±0.08	4	-7.27±0.03	89.2±2.8	-5.99±0.05	4
CL 316243	-6.03±0.04	75.8±4.5	-6.08±0.08	5	-6.17±0.12	99.8±3.8	-5.96±0.11	4
noradrenaline	-7.14±0.07	96.9±2.2	-5.83±0.08	5	-7.29±0.04	106.9±2.1	-5.89±0.04	7
adrenaline	-6.55±0.06	96.5±1.8	-5.99±.0.03	4	-6.67±0.04	109.5±2.1	-5.84±.0.08	6
isoprenaline	-7.35±0.05	100	-5.87±0.05	4	-7.46±0.05	100	-5.75±0.04	21
labetolol	-5.32±0.06	9.8±0.5	-5.53±0.08*	6	-4.97±0.09	37.4±2.1	-5.47±0.04**	5
CGP 12177	-6.57±0.09	61.4±1.5	-4.87±0.07**	5	-6.91±0.04	102.5±1.5	-5.24±0.05**	24
pindolol	-5.67±0.06	39.3±1.0	-5.29±0.05**	7	-5.69±0.05	90.1±4.0	-5.24±0.06**	8
SR 59230A	-7.21±0.04	12.3±0.8	-5.13±0.14**	6	-6.48±0.08	37.5±5.7	-5.17±0.04**	6

Log EC_{50} values, % maximal response to isoprenaline of a range of agonists and log K_D values for ICI 11855. * = $p<0.05$; ** = $p<0.01$ when comparing log K_D with that obtained in the presence of isoprenaline (ANOVA)

Baker J.G. *et al*., (2003a). *Mol. Pharmacol.* **64,** 679-688.
Baker J.G. et al., (2003b). *Mol. Pharmacol.* **63,** 1312-1321.
Konkar A.A. *et al*., (2000) *J. Pharmacol. Exp. Ther.* **294**, 923-932.