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The effect of the N-alkyl substituent on the affinity of alprenolol and oxprenolol analogues at the human β_1 and β_2 adrenoceptor (AR)

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Introduction: Alprenolol (**1a**) and oxprenolol (**2a**) are non-selective β-blockers that bind to both β_1- and β_2-ARs with high affinity. These compounds show interesting pharmacological characteristics as they can activate two agonist conformations of the β_1-AR but only one β_2-AR agonist conformation[1]. As a preliminary investigation of this phenomenon we aimed to study the effect of the amine substituent on the affinity of a series of alprenolol and oxprenolol analogues for both receptors.

Method: CHO cells stably expressing the human β_1-AR or β_2-AR were used throughout the study[2]. Ligand affinity for the β_1- and β_2-AR was assessed using ^{3}H-CGP 12177 whole cell binding. The K_D values for ^{3}H-CGP 12177 was determined by saturation binding and values of β_1 0.26 ± 0.02 nM n=10 and β_2 0.20 ± 0.02 nM n=10 obtained.

Results: All analogues bound to both receptors (Table 1). Analogues containing a primary amine (**1c/2c**) yielded the lowest affinities. Methylation of the primary amine (**1d/2d**) slightly increased affinity for β_2-AR and further homologation increased the affinity for both receptors with an ethyl substituent (**1e/2e**) as the optimal length. Branched substituents yielded the highest affinities, where analogues containing a *tert*-butyl group (**1b/2b**) improved affinity over the parent compounds (**1a-b**). Introduction of a more rigid substituent as cyclopropyl (**1h/2h**) and cyclopentyl (**1j/2j**) decreased affinity for both receptors. Interestingly, whilst all other analogues had β_2-selectivity, the addition of the 3,4-dimethoxyphenethyl group (**1l/2l**) made the compounds non-selective.

Table 1 Binding affinities for human β_1- and β_2-ARs of alprenolol and oxprenolol analogues assessed using ^{3}H-CGP 12177 whole cell binding assays. Data are mean±SEM for n experiments. **1a/2a** are the parent compounds.

Substituent (**R**)	Alprenolol analogues (**1a-l**)				Oxprenolol analogues (**2a-l**)			
	$LogK_D\beta_1$	n	$LogK_D\beta_2$	n	$LogK_D\beta_1$	n	$LogK_D\beta_2$	n
A	-7.94 ± 0.02	6	-9.01 ± 0.04	6	-7.89 ± 0.02	6	-8.77 ± 0.03	6
B	-8.51 ± 0.05	6	-9.74 ± 0.09	5	-8.37 ± 0.03	5	-9.51 ± 0.06	6
C	-6.56 ± 0.03	6	-7.18 ± 0.03	6	-6.41 ± 0.01	6	-7.07 ± 0.02	5
D	-6.58 ± 0.03	6	-7.66 ± 0.02	6	-6.44 ±0.05	6	-7.48 ± 0.04	5
E	-7.23 ± 0.02	6	-8.23 ± 0.04	6	-7.31 ± 0.04	6	-8.20 ± 0.05	5
F	-7.07 ± 0.04	6	-8.09 ± 0.11	5	-6.93 ± 0.03	6	-7.83 ± 0.04	6
G	-6.86 ± 0.02	6	-7.75 ± 0.04	6	-6.75 ± 0.03	6	-7.70 ± 0.03	5

H	-7.36 ± 0.03	6	-8.35 ± 0.06	6	-7.50 ± 0.05	5	-8.53 ± 0.04	6
I	-7.04 ± 0.02	6	-8.04 ± 0.02	6	-6.78 ± 0.06	5	-7.91 ± 0.05	5
J	-7.66 ± 0.03	6	-8.69 ± 0.03	6	-7.46 ± 0.03	5	-8.52 ± 0.08	6
K	-6.61 ± 0.04	5	-7.55 ± 0.06	5	-6.44 ± 0.04	6	-7.77 ± 0.03	6
L	-8.14 ± 0.03	6	-8.24 ± 0.04	6	-8.22 ± 0.04	6	-8.15 ± 0.04	6

Conclusion: Alprenolol and oxprenolol analogues were shown to follow the same trend. With regards to linear N-alkyl groups, ethyl substituent yielded the best affinity. In a similar manner, branching increased the affinities for both sets of analogues. Overall, the replacement of alprenolol and oxprenolol *iso*-propyl moiety by a *tert*-butyl group yielded the highest affinity for both receptors.

References:

1. Baker JG *et al.* (2003) *Mol Pharmacol* **63:** 1312–1321.

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