Poster 12

CONTRIBUTION OF TRANSMEMBRANE DOMAIN V AMINO ACIDS TO β_{1L}-ADRENOCEPTOR ACTIVITY AND AFFINITY

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There are two binding sites on the β_1-adrenoceptor (AR), β_{1H} and β_{1L} corresponding to high and low affinity binding sites respectively, which can be activated to cause cardiostimulation (reviewed Kaumann and Molenaar, 2008). Some β-blockers that block β_1AR and β_2ARs can activate β_{1L}ARs at higher concentrations than those required to cause blockade. The β_2AR does not form a corresponding low affinity binding site (Baker et al 2002) and therefore we postulated that heterologous amino acids are responsible for the formation of β_{1L}AR.

Our aim was to investigate whether heterologous amino acids of transmembrane domain V (TMDV) of β_1AR and β_2ARs contribute to β_{1L}AR. β_1ARs, β_2ARs and mutant β_1ARs containing all (β_1(β_2TMDV)AR) or single amino acids of TMDV of the β_2AR were prepared and stably expressed in Chinese Hamster Ovary cells. Concentration-effect curves for cyclicAMP accumulation were carried out for (-)-CGP12177 or (-)-isoprenaline in the absence or presence of (-)-bupranolol.

	(-)-CGP 12177	(-)-Bupranolol affinity (pK_B)	
	pEC_{50}	vs (-)-CGP 12177	vs (-)-isoprenaline
β_1AR	8.00 ± 0.11 (11)	7.23 ± 0.23 (5)	9.52 ± 0.28 (5)
β_2AR (high density)	9.24 ± 0.14 (5)	9.82 ± 0.52 (8)	
β_2AR (low density)	no effect		
β_1(β_2TMV)AR	8.86 ± 0.10 (15)	8.06 ± 0.17 (8)	9.08 ± 0.22 (6)
β_1(V230I)AR	9.07 ± 0.07 (10)	7.64 ± 0.12 (8)	9.36 ± 0.28 (9)
β_1(R222Q)AR	8.09 ± 0.29 (6)	7.33 ± 0.23 (5)	9.36 ± 0.08 (6)
β_1(V230A)AR	7.59 ± 0.09 (6)	7.32 ± 0.24 (4)	8.62 ± 0.18 (5)

The potency of (-)-CGP12177 was higher at β_2AR than at β_1AR consistent with activation through a low affinity site at the β_1AR (β_{1L}AR) but not β_2AR. The presence of V230 in β_1AR accounted for the lower potency of (-)-CGP 12177.

The affinity of (-)-bupranolol at β_1AR and mutants was higher when determined with (-)-isoprenaline than with (-)-CGP 12177. The affinity of (-)-bupranolol determined against (-)-CGP 12177 was lower at β_1AR compared to β_2AR. The presence of V230 in β_1AR accounted in part for the lower affinity.

In conclusion V230 of the β_1AR contributes in part to the low affinity binding site of β_1AR.

Baker JG, Hall IP, Hill SJ (2002). Pharmacological characterization of CGP12177 at the human β2-adrenoceptor. Br J Pharmacol 137, 400–408

Kaumann AJ, Molenaar P (2008) The low-affinity site of the β1-adrenoceptor and its relevance to cardiovascular pharmacology. Pharmacol Ther 118, 303-336