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The affinity and selectivity of orexin A, orexin B and orexin antagonists for the human orexin 1 and orexin 2 receptors.

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Introduction. Orexin A and orexin B are endogenous wake-promoting neuropeptides that activate orexin 1 (Ox1R) and orexin 2 (Ox2R) receptors and a lack of orexin leads to narcolepsy [1]. Orexin agonists are a potential novel treatment for narcolepsy while orexin antagonists may be useful for insomnia. Here we investigate the affinity of several compounds reported to be orexin receptor ligands using 3 different radioligands.

Method. CHO cells stably expressing the human Ox1R or Ox2R were used and whole cell radioligand binding assays performed using ^{3}H-SB674042, ^{3}H-EMPA or ^{3}H-almorexant (2hr, 37°C). Suvorexant (10μM) was used to determine non-specific binding. Radioligand K_D values were obtained from saturation experiments and K_D values for competing ligands were calculated using the Cheng-Prusoff equation [2].

Results. Saturation binding revealed an affinity for ^{3}H-SB674042 of 4.65±0.28nM (n=15) for the Ox1R, but was too low to determine for the Ox2R. The affinity of ^{3}H-EMPA was 7.86±0.46nM (n=12) for the Ox2R, but was too low to determine for the Ox1R. The affinity of ^{3}H-almorexant was 1.72±0.27nM (n=13) for the Ox1R and 2.68±0.53nM (n=12) for the Ox2R. The experimental window (difference between total and non-specific binding) was however considerably larger for ^{3}H-SB674042 (Ox1R) or ^{3}H-EMPA (Ox2R) than that for ^{3}H-almorexant (Figure 1). Although most ligands fully inhibited specific binding to yield log K_D values (Table 1, Figure 2), orexin A and orexin B only partially inhibited the specific binding of ^{3}H-SB674042 and ^{3}H-EMPA at both Ox1R and Ox2R to yield apparent K_D values as shown (Figure 2, Table 1).

Table 1. Log K_D values (mean ± sem of n separate experiments) and selectivity values for ligands binding to the human orexin1 and orexin2 receptors stably expressed in CHO cells. Thus the affinity of SB334867 is 64.6 times higher for the orexin1 receptor than the orexin2 receptor (as taken from ^{3}H-SB674042 and ^{3}H-EMPA competition binding).

app = apparent KD value. Orexin A and B did not fully inhibit the radioligand specific binding (see Figure 2) and thus apparent log K_D values are given.

ND = not determined. Unable to accurately determine due to small specific binding window

	CHO-Orexin 1				CHO-Orexin 2				selectivity	
	^{3}H-SB674042		^{3}H-almorexant		^{3}H-EMPA		^{3}H-almorexant		Ox1	Ox2
Orexin A	-7.32 ± 0.07^{app}	9	-7.34 ± 0.18^{app}	7	-6.88 ± 0.08^{app}	6	ND		2.8	
Orexin B	-6.16 ± 0.09^{app}	8	-6.36 ± 0.08^{app}	6	-7.30 ± 0.08^{app}	6	ND			13.8
SB334867	-7.34 ± 0.04	6	-7.46 ± 0.04	6	-5.53 ± 0.03	6	-5.01 ± 0.05	5	64.6	
SB674042	-8.37 ± 0.05	7	-8.44 ± 0.09	8	-6.87 ± 0.09	5	-5.67 ± 0.07	8	31.6	
ACT335827	-7.45 ± 0.05	7	-7.34 ± 0.11	7	-6.24 ± 0.05	5	-5.08 ± 0.05	7	16.2	
SB408124	-6.68 ± 0.07	7	-6.68 ± 0.13	7	-5.50 ± 0.05	6	IC50 >-4	8	>15.1	
Suvorexant	-8.62 ± 0.04	13	-8.74 ± 0.08	12	-8.53 ± 0.09	7	-7.67 ± 0.06	13	1.2	
Almorexant	-8.31 ± 0.07	7	-8.20 ± 0.15	7	-8.72 ± 0.05	7	-8.35 ± 0.09	7		2.6
TCS1102	-8.12 ± 0.10	6	-8.04 ± 0.13	6	-8.66 ± 0.06	7	-7.87 ± 0.09	6		3.5
IPSU	-6.50 ± 0.05	7	-6.43 ± 0.13	7	-7.45 ± 0.09	8	-6.32 ± 0.05	7		8.9
ACT462206	-7.29 ± 0.06	9	-7.31 ± 0.08	6	-8.29 ± 0.07	5	-7.17 ± 0.04	8		10.0
TCSox229	-5.29 ± 0.05	7	-5.34 ± 0.09	7	-7.00 ± 0.04	6	-6.03 ± 0.02	8		51.3
JNJ10397049	-5.94 ± 0.03	6	-5.79 ± 0.11	5	-8.36 ± 0.04	7	-7.46 ± 0.08	6		263
EMPA	-5.58 ± 0.05	10	-5.40 ± 0.13	9	-8.22 ± 0.03	6	-7.26 ± 0.06	11		437
Primaquine	IC_{50}>-4	5	No binding	5	IC_{50}>-4	3	No binding	5		
Carvedilol	-5.07 ± 0.07	5	IC_{50}>-5	5	-5.15 ± 0.02	6	IC_{50}>-5	5		

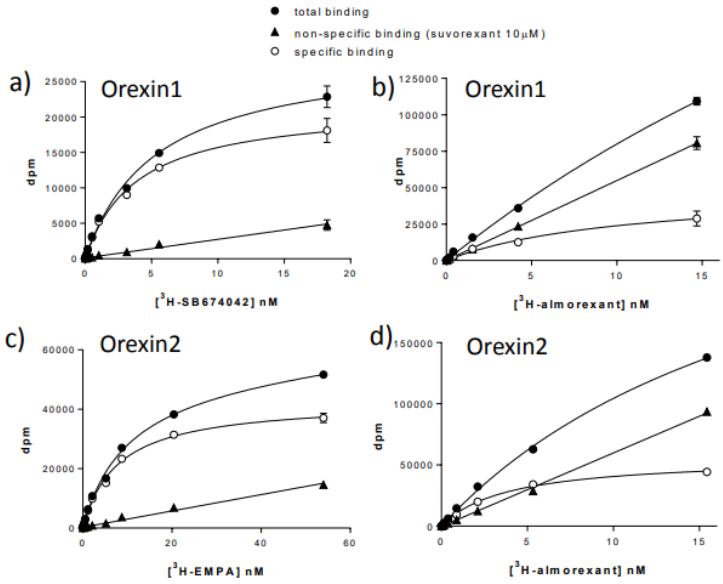

Figure 1.
^{3}H-whole cell radioligand saturation binding to human orexin1 and orexin2 receptors stably expressed in CHO cells with a) ^{3}H-SB674042 (orexin1), b) ^{3}H-almorexant (orexin1), c) ^{3}H-EMPA (orexin2), and d) ^{3}H-almorexant (orexin2). Data points are mean ± sem of quadruplicate determinations and each experiment is representative of a) 15, b) 13, c) 12 and d) 12 separate experiments.

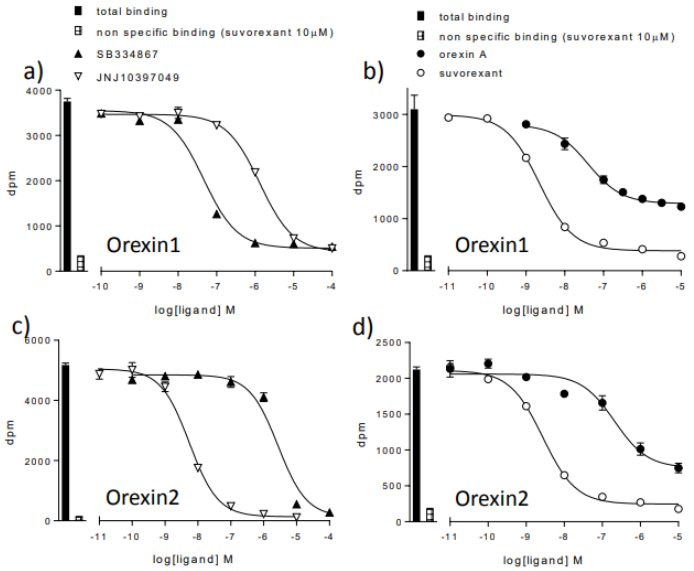

Figure 2.
Inhibition of ^{3}H-SB674042 (orexin1, a and b) and ^{3}H-EMPA (orexin2, c and d) whole cell binding in CHO cells expressing the human orexin1 and orexin2 receptors. The concentration of radioligand is a) 0.59nM, b) 0.58nM, c) 0.83nM, d) 0.50nM, Data points are mean ± sem of triplicate determinations and each experiment is representative of a) 6, b) 9, c)6 and d) 7 separate experiments.

Conclusion. Ligands with Ox1R selectivity (e.g. SB334867 and SB674042) and Ox2R selectivity (e.g. EMPA and JNJ10397049) were identified as well as high affinity non-selective ligands (e.g. almorexant and

suvorexant). The larger window of specific binding made ^{3}H-SB674042 and ^{3}H-EMPA better radioligands than ^{3}H-almorexant in this whole cell binding assay. Primaquine and carvedilol, previously reported to have orexin receptor affinity [e.g. 3], had no, or extremely poor affinity only. Whereas most ligands fully inhibited the radioligand specific binding (suggesting competition at the same binding site), orexin A and B were only able to partially displace ^{3}H-SB674042 (Ox1R) and ^{3}H-EMPA (Ox2R) specific binding suggesting that the binding sites of orexin A and B do not fully overlap with those of the radioligands.

References.

[1] Gotter AL et al., 2012 *Pharmacol Rev* **64:** 389-420
[2] Baker JG (2005) *Br J Pharmacol*. **144:** 317-322.
[3] Turku et al., 2016 *J Med Chem* **59:** 8263–8275