Serotonin Receptor Profiles of Bedtime Pharmacotherapies Targeting Posttraumatic Stress Disorder (PTSD)

Abstract 728

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Introduction

- Syndromal sleep disturbances in PTSD are targeted by drugs that antagonize serotonin (5-HT) receptors, particularly 5-HT_{2A} and 5-HT_{2C}[1]
- Several lines of evidence implicate antagonism of 5-HT_{2A} and 5-HT_{2C} receptors in the enhancement of slow wave sleep (SWS), the type of sleep often referred to as restorative sleep[1]
- Cyclobenzaprine (CBP) and trazodone (TZD) are bedtime PTSD treatment candidates with several 5-HT receptor-mediated actions, and both have major metabolites that are differentially active at 5-HT receptors
  - meta-chlorophenylpiperazine (mCPP), the major metabolite of TZD, at 1 mg/kg i.v. produces flashbacks, panic attacks and exacerbates other PTSD symptoms in about a third of patients with combat PTSD[2]
- In this work, the activities of CBP and TZD, and their respective metabolites norcyclobenzaprine (nCBP) and mCPP, on human 5-HT receptors were investigated

Methods

Radiolabeled binding assays

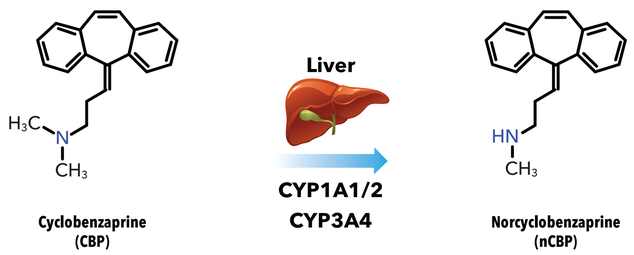
- Receptor binding assays were performed under equilibrium conditions on Chinese hamster ovary (CHO) cell membranes expressing the various recombinant human receptors
- Binding of ^{3}H-labeled ligands specific for each receptor were carried out in the presence of varying concentrations of unlabeled compounds using standard procedures (Eurofins Scientific, France)
- Inhibition constants (K_i) were calculated using the Cheng-Prusoff equation ($K_i = IC_{50}/(1+L/K_d)$), where L = concentration of radioligand, and K_d = affinity for the receptor

Ligand-induced calcium mobilization assays

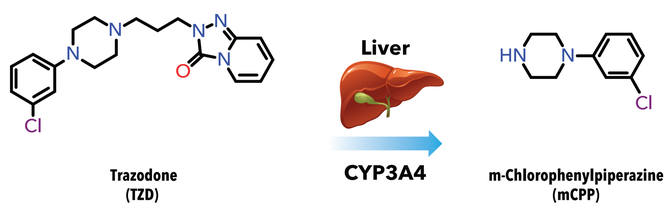
- Rat basophilic leukemia (RBL) cells expressing the various recombinant human receptors were evaluated for agonist and antagonist activity of the various compounds in ligand-induced calcium mobilization using standard procedures (Eurofins Scientific, St. Charles, MO)
- Maximum values were converted to percent activation (relative to reference agonist and vehicle control values) and percent inhibition (relative to vehicle control values)

The Liver Transforms Cyclobenzaprine and Trazodone to Active Metabolites

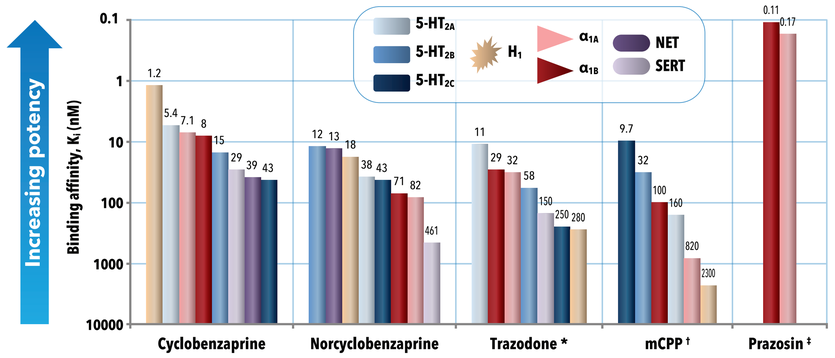
Cyclobenzaprine (CBP) is metabolized by hepatic p450 isoforms into the active metabolite norcyclobenzaprine (nCBP)



Trazodone (TZD) is metabolized by hepatic p450 isoforms into the active metabolite meta-chlorophenylpiperazine (mCPP)

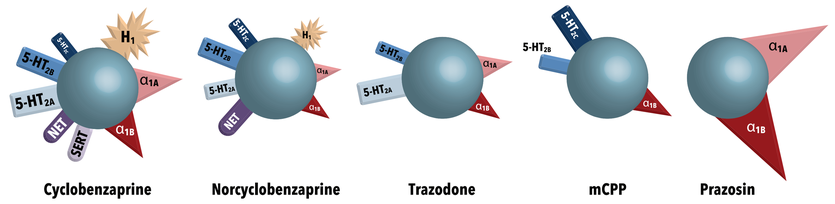


Cyclobenzaprine Has Moderate to High Binding Affinities on Multiple Receptors



* Trazodone was not active on NET
† mCPP was not tested on NET or SERT
‡ Prazosin was not active on 5-HT_{2A}, NET, H_1 or SERT. It was not tested on 5-HT_{2B} or 5-HT_{2C}

Cyclobenzaprine Shows a Balanced Binding Profile



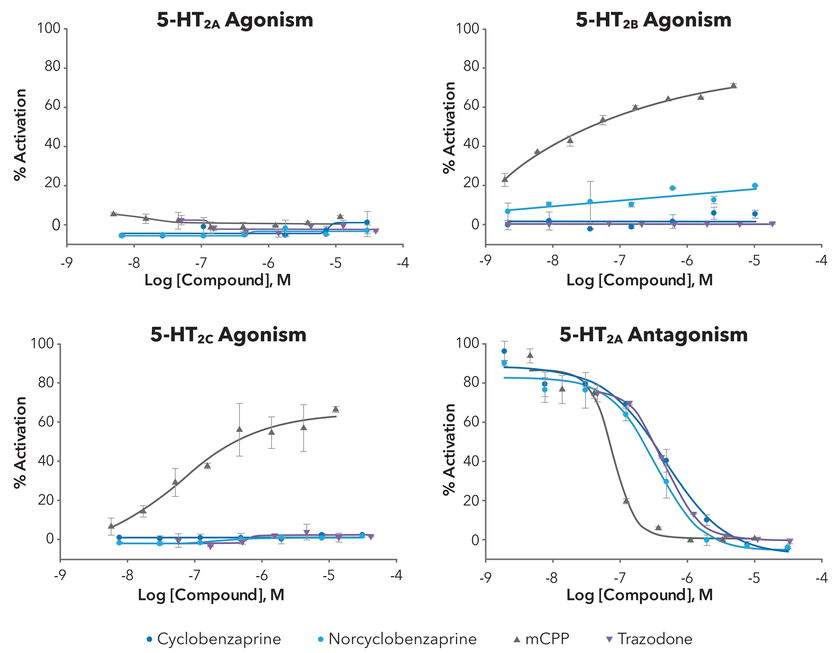
- The size of the receptor icon is proportional to its activity on that receptor
- Receptor icons are shown for K_i's of <100 nM
- Cyclobenzaprine has potent activity on multiple receptors
- Norcyclobenzaprine binds to the same receptors as cyclobenzaprine, although with less potency

Cyclobenzaprine is a Functional Antagonist on Putative Sleep Receptors

Receptor	CBP	nCBP	TZD	mCPP	Prazosin
5-HT_{2A}	230	140	470	79	-
5-HT_{2B}	100	580	3000	6.2	-
5-HT_{2C}	444	1220	*No Activity*	75	-
H_1	5.2	16	-	-	-
α_{1A}	4.9	16	34	1100	1.0
α_{1B}	530	790	360	2200	0.64
NET	39	32	-	-	-
SERT	420	2000	-	-	-

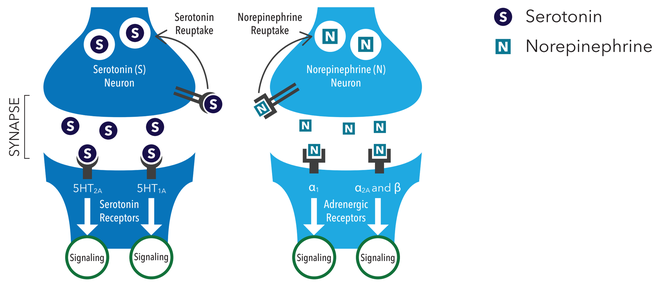
- Antagonist values are reported as half-maximal inhibitory concentration (IC_{50}). Agonist values (in red) are reported as half maximal effective concentration (EC_{50}).

meta-Chlorophenylpiperazine is an Agonist on 5-HT_{2B} and 5-HT_{2C} Receptors

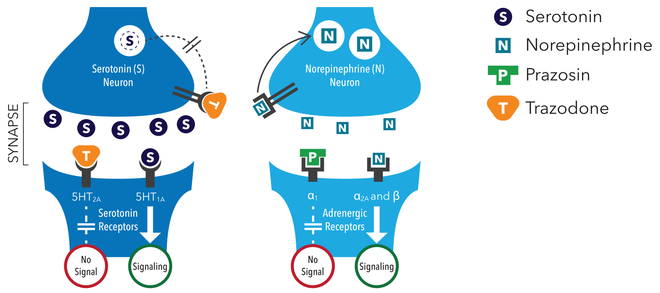


Cyclobenzaprine Can Act Through Dual Signaling Pathways

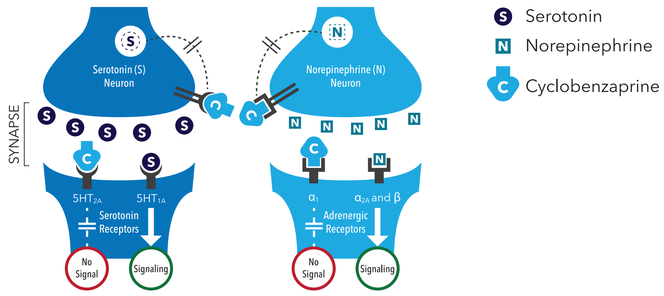
Signaling in Neurons



Effects of Trazodone and Prazosin



Cyclobenzaprine Combines Activities of Trazodone & Prazosin, Plus NET Inhibition



Conclusions

Cyclobenzaprine is a Serotonin and Norepinephrine receptor Antagonist and Reuptake Inhibitor (SNARI)

- CBP has potent antagonist activity at 5-HT_{2A}, α_{1A}, and H_1 receptors
- 5-HT_{2A} antagonist activity of CBP is in common with TZD, commonly used for sleep effects in psychiatric conditions, including off-label use in PTSD
- α_{1A} antagonist activity of CBP is in common with prazosin, commonly used off-label to treat night terrors and sleep disturbance in PTSD
- CBP is metabolized into the active metabolite nCBP, which is a stronger NET inhibitor and has a similar binding profile to CBP, albeit with less potency
- TZD is metabolized into the active metabolite mCPP, an *agonist* at 5-HT_{2C} (suspected to cause panic- and flashback-inducing effects in combat PTSD[2])
- The lack of 5-HT_{2C} agonist effects of it or its metabolite makes CBP a promising candidate for clinical trials of bedtime therapy targeting sleep disturbance for improving daytime symptoms of PTSD
- As noted by Jonathan Davidson, "Opportunities exist to reassess the efficacy and safety of TCAs [for PTSD]…Examples in support of this contention include the use of low-dose…cyclobenzaprine"[3]
- Tonix is currently conducting a Phase 2 study to investigate the efficacy and safety of low-dose, sublingual CBP for the treatment of military-related PTSD (ClinicalTrials.gov Identifier: NCT02277704)

References

1. Landolt HP, Wehrle R. Antagonism of serotonergic 5-HT2A/2C receptors: mutual improvement of sleep, cognition and mood? *Eur J of Neurosci.* 2009;29:1795–1809.
2. Southwick SM, Krystal JH, Bremner JD, et al. Noradrenergic and serotonergic function in posttraumatic stress disorder. *Arch Gen Psychiatry.* 1997;54:749-758
3. Davidson J. Vintage treatments for PTSD: a reconsideration of tricyclic drugs. *J Psychopharmacol.* 2015;29:264-9.

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