Dexmedetomidine Reduces Stress-Related Behaviors and Improves Sleep Parameters in Rodent Models

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INTRODUCTION

- BioXcel Therapeutics is developing BXCL501, an orally dissolving, thin film formulation of dexmedetomidine, that contains the drug dexmedetomidine. BXCL501 reduced agitation in patients with schizophrenia and bipolar disorder in 2 separate Phase 3 studies.
- Dexmedetomidine is a highly potent and selective agonist at the alpha2-adrenergic receptor (ADRA2), a class of drugs that includes clonidine, lofexidine, and guanfacine.
- Stress activates the locus coeruleus (LC) and increases noradrenergic signaling resulting in changes in behaviors.[1] Activation of ADRA2 reduces LC activity.[2]
- Dexmedetomidine has poor oral bioavailability and is not useful for oral ingestion.[3] Administration by sublingual (SL) or intramuscular (IM) routes avoids first pass metabolism and the full therapeutic value of dexmedetomidine may be achieved.
- In this study, we show that IM administration of dexmedetomidine to rats reduces stress-mediated behaviors in the forced swim test, a task associated with LC hyperactivation.[4] In addition, dexmedetomidine has a favorable effect on the sleep hypnogram. Sleep architecture is also related to LC activity.

METHODS

In Vitro Potency and Intrinsic Activity
$GTP\gamma S$ Assay: Cells were transfected with human alpha2-adrenergic receptors (A, B and C). Drug-stimulated $GTP\gamma S$ activity was measured and used to construct concertation-response curves as described in Audinot et al.[5] EC_{50} vales and intrinsic activity (relative to the positive control) were calculated using a 3-parameter logistic equation and fitted using GraphPad Prism.

Microdialysis: Ten male Sprague Dawley rats with a jugular vein (JV) cannula were used in this study. Animals were placed in a stereotaxic frame (Kopf instruments, USA) and MetaQuant microdialysis probes (PAN membrane, BrainLink, Netherlands) were implanted into the prefrontal cortex (PFC), 4 mm exposed surface. Coordinates for the tips of the probes for the PFC are: antero-posterior (AP) = +3.4 mm from bregma, lateral (L) = -0.8 mm from midline and ventral (V) = -5.0 mm from dura, the toothbar set at -3.3 mm. Microdialysis was performed approximately 24 h following the surgery. Microdialysis samples were collected for 30 minutes. In addition to ISF collection, blood samples were collected by a jugular vein cannula and processed for plasma by centrifugation. Study was performed by Charles River Laboratories in South San Francisco.

Forced Swim Test: Male Sprague Dawley rats (240 g, 8 weeks old) were used. All rats were exposed to a swim test prior to compound administration. On Day 2, rats were administered vehicle or dexmedetomidine, 1 or 5 mcg/kg intramuscular (IM) gastrocnemius muscle, 1 hour prior to test. Each behavior was measured during a 5 second interval and then summed over 5 minutes. Data were presented as a frequency of total behavior for immobility, climbing, and swimming. Study was performed at Psychogenics Inc (Paramus, NJ)

Locomotor Activity Test
After completion of the FST, the same rats that were tested in the locomotor activity test. Distance traveled was measured from horizontal beam breaks as the rat moved, whereas rearing activity was measured from vertical beam breaks. On the day of testing, animals were acclimated in the experimental room for at least 1 h prior to administering vehicle or dexmedetomidine, 1 or 5 mcg/kg IM (gastrocnemius muscle). Locomotor activity was recorded for 30 minutes.

REM and Slow Wave Sleep
Sleep-EEG and Data Analysis
Rats were tested twice weekly in a cross over design with a minimum of 3-day washout period between doses. Four sleep stages were defined: Active Wake; Quiet Wake; NREM (Slow Wave) sleep, and paradoxical or REM sleep. Fast Fourier Transform was used for spectral analysis. Fast Fourier Transform of the EEG signal showed that the increase in slow wave sleep was matched by a dose and time-dependent increase in delta frequencies (0.5-6Hz). Spectral power was measured for each 10s epoch at 1Hz resolution (1-100 Hz) and summed to yield cumulative power. Study was performed at Psychogenics Inc (Paramus, NJ).

RESULTS

In Vitro Potency & Brain Levels

Pharmacology at Alpha2-Adrenergic Receptors

Drug	ADRA2A		ADRA2B		ADRA2C	
	EC_{50} (nM)	Max activity	EC_{50} nM	Max activity	EC_{50} nM	Max activity
Dexmedetomidine	3.9	89	2.8	147	14	110
Clonidine	25	76	49	49	43	32
Guanfacine	69	78	2010	110	45	72
Lofexidine	17	40	43	97	23	50

TABLE 1. Potency and activity of clinically useful alpha2-adrenergic agonists were determined by using agonist-stimulated $GTP\gamma S$ binding to cells transfected with human alpha2A, 2B or 2C adrenergic receptors. Maximum activity is expressed as percentage of the maximal activity achieved by the positive control.

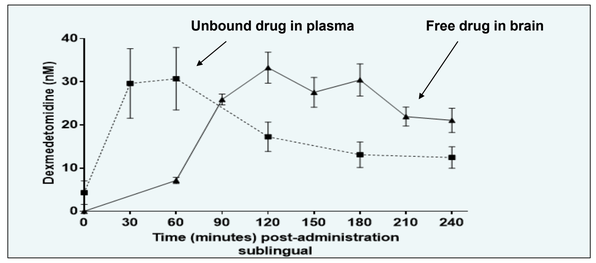

Figure 1. Rats were dosed with 1000 mcg/kg of dexmedetomidine (sublingual). Plasma and free brain concentrations of drug were measured using microdialysis probes at the indicated time points. Free plasma (unbound drug) was calculated by adjusting total plasma assuming that drug is 90% bound.

Effects of IM Dexmedetomidine on Stress-Related Behavior: The Forced Swim Test

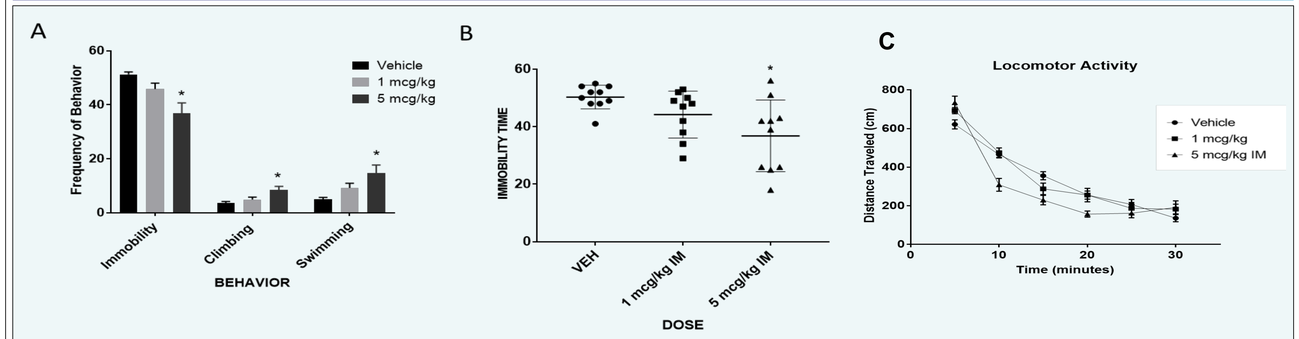

Figure 2. Forced swim test is a task used to test behavioral response to stress. Dexmedetomidine at 5 mcg/kg (IM) effectively reduced immobility time (A and B) and increased the frequency of escape behaviors (swimming and climbing) but did not increase locomotor activity (C).

Effects of IM Dexmedetomidine on Sleep

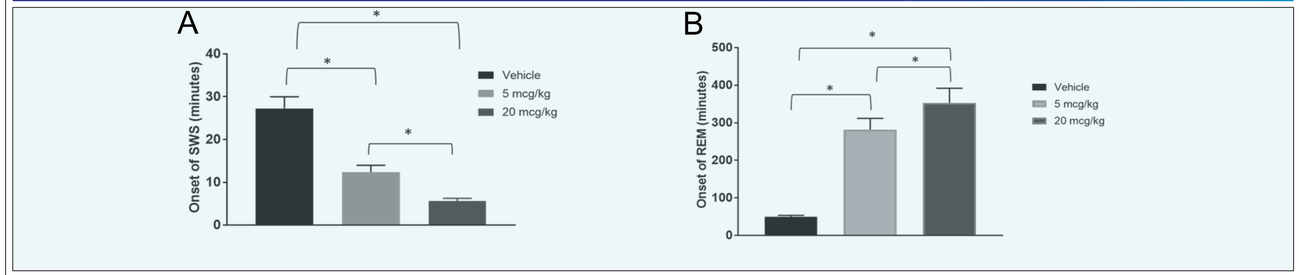

Figure 3. Dexmedetomidine effectively reduced latency to slow wave sleep (A) and increased latency to REM sleep (B).

SUMMARY & DISCUSSION

- Dexmedetomidine is more potent and has higher intrinsic activity at the ADRA2s in vitro than other clinically used alpha2 adrenergic receptor agonists (Table 1)
- Dexmedetomidine (unbound drug) is freely permeable between plasma and brain (Figure 1)
- Dexmedetomidine decrease immobility time and increase escape behaviors (Figures 2A and B), without increasing locomotor activity (Figure 2C) in the forced swim test. Benzodiazepines increase immobility time in this model.[6]
- Dexmedetomidine increased latency to REM sleep, a clinically useful translatable biomarker of CNS activity, and decreased latency to slow wave sleep (Figures 3A and B). GABAergic drugs display an inferior profile compared to dexmedetomidine with respect to slow wave sleep.[7]
- Effects on both behavior and sleep occurred at doses 1/200th of those evaluated by microdialysis indicating that likely free brain levels at efficacious doses were less than 0.15 nM (occupancy of less than 10% of receptors assuming an EC_{50} of 3 nM).
- In Phase 3 clinical studies, BXCL501 produces plasma exposures at effective doses in patients nearly identical to those reported in these studies.[8]

CONCLUSIONS

- Dexmedetomidine affects behaviors related to LC activity: the FST and sleep architecture. Plasma exposures at effective doses are consistent with low (<10%) occupancy of the ADRA2. Because only a small percentage of receptors are occupied at therapeutic doses, we predict that chronic dosing will be unlikely to desensitize ADRA2-mediated response.
- Dexmedetomidine's effects on escape behaviors and sleep differ from benzodiazepines and therefore this drug represents a novel approach for the treatment of symptoms associated with psychiatric and neurological diseases.

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