



THE ANXIOLYTIC COMPOUND BNC210 EXHIBITS AN ANTIDEPRESSANT EFFECT WITHOUT SYMPTOMS OF PHYSICAL DEPENDENCE

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Introduction

BNC210 is a novel compound that displays activity in several models of anxiety across three rodent species. BNC210 is free of sedative side effects and does not cause abuse liability or development of tolerance. We have investigated the potential antidepressant activity of BNC210 in the rat Forced Swim Test which is used to measure the effect of antidepressant drugs on the behaviour of rodents. Clinically active antidepressants delay the onset of immobility in this assay and reduce the total time of relative immobility. In addition, we evaluated the potential for development of physical dependence to BNC210 treatment. Abrupt cessation of antidepressant medication may produce withdrawal effects seen as in changes in body weight, food intake or body temperature. Such changes signify physical dependence to the drug. Furthermore, antidepressants increase neurogenesis in various brain regions of rodents, nonhuman primates and humans (Boldrini et al, 2009 *Neuropsychopharmacology*, **34**, 2376-2389) and induce neurite outgrowth *in vitro* (Fricker et al, 2005, *Brain Res Mol Brain Res*, **38**, 228-235). Given its antidepressant activity, the ability of BNC210 to induce neurite outgrowth in primary rat cortical neurons was investigated. The data presented demonstrate that BNC210 acts as an antidepressant without the liability of physical dependence and has the ability to enhance neurite outgrowth in primary neurons. These observations provide strong evidence for the potential utility of BNC210 in the treatment of depression.

Methods

FORCED SWIM TEST

The rat forced swim test (FST) is the most widely used paradigm for evaluation of the potential antidepressant effect of drugs (Porsolt et al, 1978 *Eur. J. Pharmacol.* **47**:379-391). Clinically active antidepressants have been found to reduce the total time of relative immobility. This test is performed for 5 minutes in a glass cylinder (35 cm High; 24 cm Diameter) containing water to a depth of 25 cm and maintained at a temperature of 25±1°C. BNC210 was administered to rats PO, 60 minutes prior to the test and the tricyclic antidepressant Imipramine (comparator) was administered IP, 30 minutes prior to the swim test. The effect of each drug on immobility time was measured.

NON-PRECIPIATED WITHDRAWAL TEST

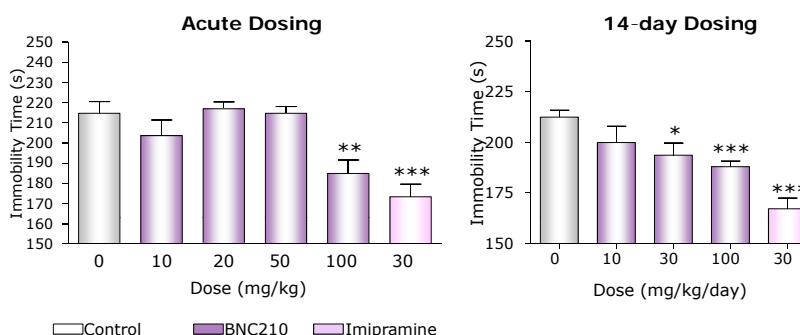
The aim of this assessment was to evaluate whether sudden cessation of chronic drug treatment was associated with the occurrence of identifiable physical signs of withdrawal such as changes in body weight, food intake and body temperature. This method (Goudie et al, 1993 *NeuroReport* **4**:295-299) can be used to evaluate a wide variety of substances including those for which specific antagonists are not available. Rats received daily doses of BNC210, (0, 10, 30 and 100 mg/kg, PO) for 14 consecutive days. In the 4-5 day period following drug withdrawal, daily recordings of food intake, body weight and body temperature were made.

NEURITE OUTGROWTH

Commonly used antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) have been shown to potentiate neurite outgrowth in primary neuronal cultures. Primary rat cortical neurons were seeded into poly-L-lysine coated Petri dishes (ø 35mm) at a density of 30,000 cells / dish. After neuronal adhesion, cultures were exposed to BNC210 or Brain Derived Neurotrophic Factor (BDNF) for 3 days. BNC210 was tested at 0.1 nM, 1 nM, 10 nM, 100 nM and 1000 nM on two independent cultures comprising 2 Petri dishes per culture and per condition. BDNF was tested in parallel at 50 ng/mL. After 3 days exposure to the test compounds, cells were washed and fixed. Approximately 90 images of neurons with neurites without any branching were taken per condition (n=180). Neurite length was automatically calculated using imaging software (Image-Pro Plus, France).

Results

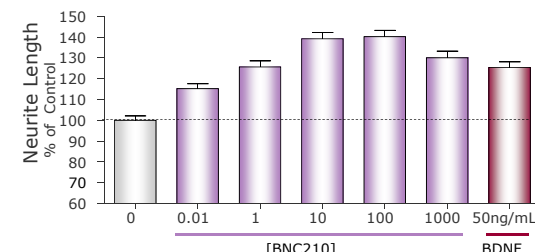
BNC210 EXHIBITS ANTIDEPRESSANT ACTIVITY IN THE RAT FORCED SWIM TEST



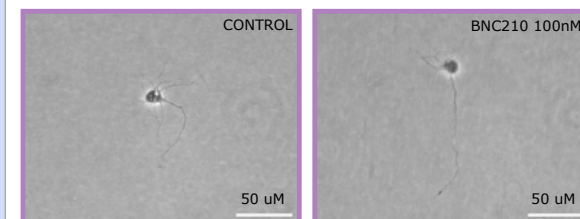
ACUTE DOSING: BNC210 was administered to rats at 0, 10, 20, 50 and 100 mg/kg 1 hour prior to exposure to the FST. Significantly reduced immobility time compared to the vehicle treated rats was observed for the 100 mg/kg dose. The lower doses of BNC210 were not active.

14-DAY REPEAT DOSING: BNC210 was administered to rats at 0, 10, 30 and 100 mg/kg/day for 14 days. Exposure to the FST occurred 1 hour after dosing on day 14. Chronic administration produced augmentation of the antidepressant effect of BNC210 which was demonstrated by a significant reduction in immobility time in rats treated with both 30 and 100 mg/kg/day. *p≤0.05; **p≤0.01; ***p≤0.001; Significantly different to vehicle control; Unpaired T-test. n=10 rats

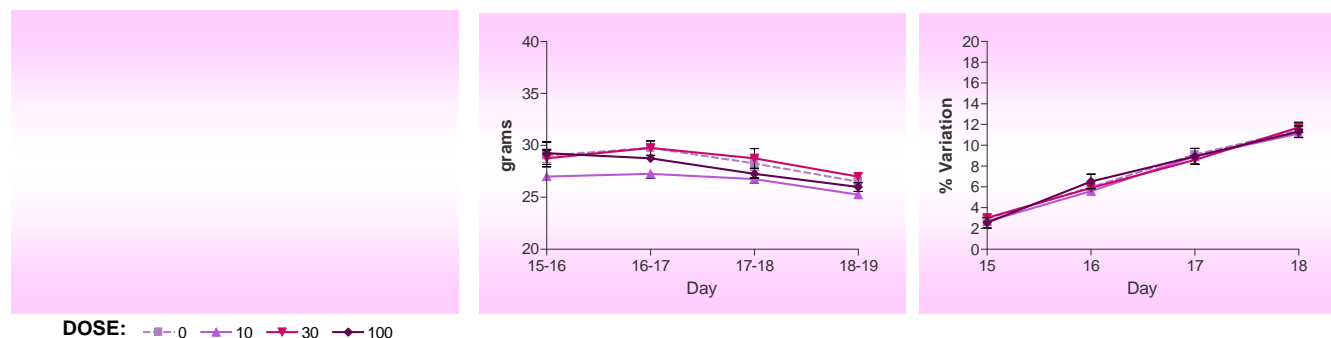
BNC210 IS A POTENT ENHANCER OF NEURITE OUTGROWTH IN PRIMARY CORTICAL NEURONS



All concentrations tested had a significant effect. The magnitude of response with 1 nM of BNC210 was similar to that produced by BDNF (50 ng/mL), while concentrations in the range of 10 to 1000 nM were more effective than BDNF. The concentrations at which BNC210 exerts its effects in this system correlate with its *in vivo* potency and measured brain concentrations. ANOVA followed by Tukeys Multiple Comparison Test** p≤0.01; *** p≤0.001 significantly different to vehicle control; n ~ 180 cells



BNC210 DOES NOT PRODUCE SIGNS OF WITHDRAWAL FOLLOWING A 14-DAY DOSING PERIOD



Rats treated chronically with opioids, benzodiazepines or SSRIs display adverse physical effects after non-precipitated withdrawal of the drugs. We assessed the potential consequences of abrupt cessation of dosing with BNC210 following 14 days of treatment at 0, 10, 30 and 100 mg/kg/day. Withdrawal of BNC210 treatment did not produce significant changes in body temperature, weight gain or food consumption compared to the no-drug treatment group during the post-treatment period (5 days). These findings indicate that repeat dosing with BNC210 does not cause the development of physical dependence to the drug and is consistent with its suitability for chronic use. n=10 rats

Conclusions

- ◆ The antidepressant profile of BNC210 is consistent with clinically active antidepressants:
 - A: BNC210 exhibits antidepressant activity in the rat forced swim test - doses of BNC210 reduce the total time of relative immobility and show comparable efficiency to Imipramine
 - B: The antidepressant potency of BNC210 is markedly increased following 14-days of repeat administration demonstrating that sensitization to the effect of BNC210 has developed
 - C: BNC210 enhances neurite outgrowth in primary cortical neurons.
- ◆ In contrast to antidepressants and other anxiety medications, withdrawal of BNC210 treatment following 14-day repeat dosing does not result in the adverse physical events associated with physical drug dependence.
- ◆ The profile of BNC210 in these preclinical experiments supports expansion of its therapeutic potential to the treatment of generalized anxiety disorder with co-morbid depression.
- ◆ The safety and pharmacokinetics of BNC210 in healthy volunteers is currently being evaluated in a Phase I clinical trial.