The phospholipase C inhibitors U73122, D609 and Edelfosine inhibit the activity of the novel anxiolytic and antidepressant compound BNC210

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NEUROFIT PRECLINICAL RESEARCH

INTRODUCTION

BNC210 is a novel compound with potent activity in animal models of anxiety and depression. In common with other antidepressant compounds, BNC210 has demonstrated potent enhancement of neurite outgrowth in rat primary cortical neurons. Doses ≥1 nM produce a BDNF-equivalent response in this system. One pathway which is critical for the effect of BDNF on synaptic plasticity involves signalling through Trk receptors via phospholipase C gamma-1 (PLC γ-1). PLC enzymes also form part of the intracellular signalling pathway for neurotransmitters binding to G-protein-coupled receptors (GPCR), many of which demonstrate neurotrophic properties.

AIMS

The purpose of this study was to elucidate the involvement of PLC enzymes in the neurotrophic and anxiolytic effects of BNC210 using PLC-specific inhibitors:

- U73122 (inhibitor of phosphotidyl-choline PLC (PC-PLC) and phosphotidyl-inositol PLC (PI-PLC) enzymes
- D609, a PC-PLC specific inhibitor
- Edelfosine (ET-18-O-CH3), a PI-PLC specific inhibitor

MATERIALS AND METHODS

Neurite Outgrowth Assays: Rat primary cortical neurons from E17 rat fetuses were treated with 10 nM BNC210 plus/minus U73122. Following cell attachment, U73122 (30–1000 nM) was added to the cultures for 1 hour prior to BNC210 addition. Neuronal cultures were exposed to test compounds for three days and then washed and fixed. Photographs of neurons with neurites, without any branching, were taken from each condition (n~180). Neurite length was calculated using Image-Pro Plus software.

Mouse Light/Dark Box (LD Box): The time spent, number of transitions and total walked distance in the lit box was recorded over a 5 minute period. Mice were dosed by oral gavage with BNC210 or Diazepam 60 minutes prior to testing. U73122 and Edelfosine were administered two hours prior to testing [1, 3]. D609 was administered one hour prior to testing [2].

CONCLUSIONS

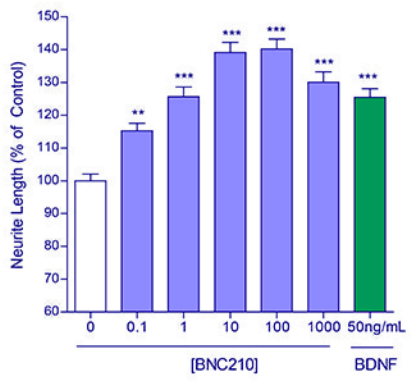
U73122 gave full block of BNC210's effects in the neurite outgrowth assay.
U73122 and **D609** also produced full block of BNC210 in the mouse LD Box, without affecting the anxiolytic activity of Diazepam, suggesting that the effect of these PLC blockers was BNC210 specific and not a general 'anti-anxiolytic' effect.
Similarly, in animals pre-treated with **Edelfosine**, the anxiolytic activity of BNC210 was abolished while Diazepam treatment fully reversed the anxiogenesis induced by Edelfosine.
This work suggests that BNC210 signalling networks involve PI- and PC-PLC enzymes and implicate GPCRs or Trk receptors in the mediation of BNC210's effects on neurite outgrowth and regulation of mood disorders.

REFERENCES

1. Hou, 2004. JPET May; 309(2): 697–704.
2. Ansari, 2006. Free Radic Biol Med. December 1; 41(11): 1694–1703.
3. Mollinedo, 2010. Clin Cancer Res; 16(7): 2046-2054.

RESULTS: NEURITE OUTGROWTH

A:BNC210 and BDNF



B:BNC210 and U73122

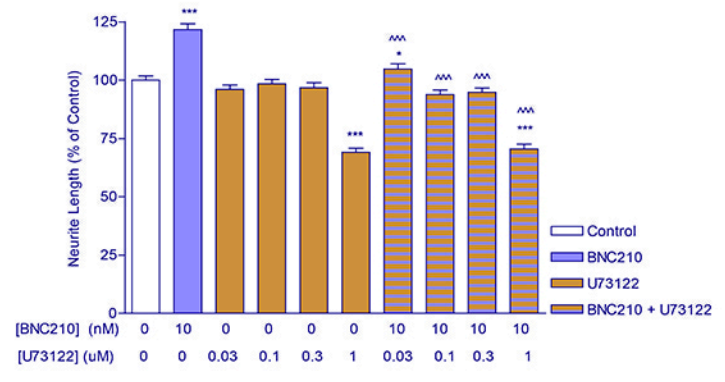


Figure 1:
(A) BNC210 (0.1-1000 nM) significantly enhanced neurite outgrowth. Concentrations from 1-1000 nM produced increases in neurite length that were ≥ BDNF (50 ng/mL) responses.
(B) U73122 caused a dose-dependent inhibition of BNC210's (10 nM) effect, with doses of 0.1–1 uM producing full block.

RESULTS: MOUSE LIGHT DARK BOX

ENTRIES TIME DISTANCE

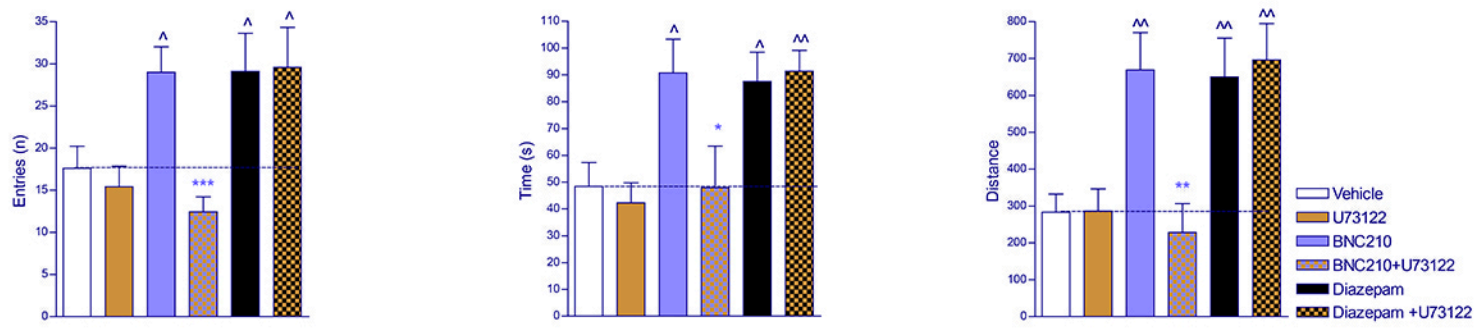


Figure 2: U73122
BNC210 (10 mg/kg; p.o.) and Diazepam (1 mg/kg; p.o.) demonstrated similar efficacy and significant anxiolytic activity in this model. U73122 (30 mg/kg; i.p.) treatment alone did not affect mouse behaviour. However, when BNC210 and Diazepam were administered to mice pre-treated with U73122, the anxiolytic activity of BNC210 was abolished while the effect of Diazepam was unchanged.

ENTRIES TIME DISTANCE

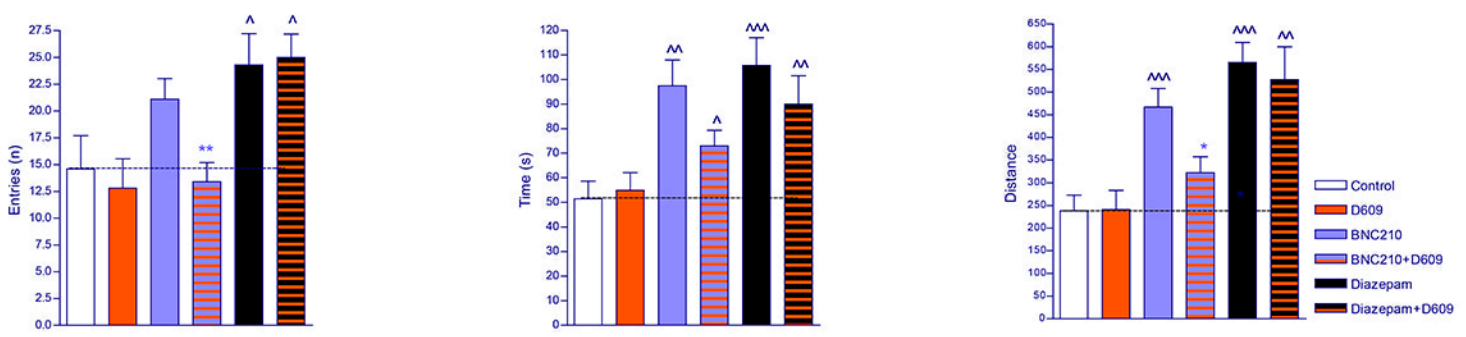


Figure 3: D609
BNC210 (10 mg/kg; p.o.) and Diazepam (1 mg/kg; p.o.) demonstrated significant anxiolytic activity in this model while D609 (50 mg/kg; i.p.) alone had no effect on mouse behaviour. When BNC210 and Diazepam were administered to mice pre-treated with D609, the anxiolytic activity of BNC210 was significantly reduced while the effect of Diazepam was unchanged.

ENTRIES TIME DISTANCE

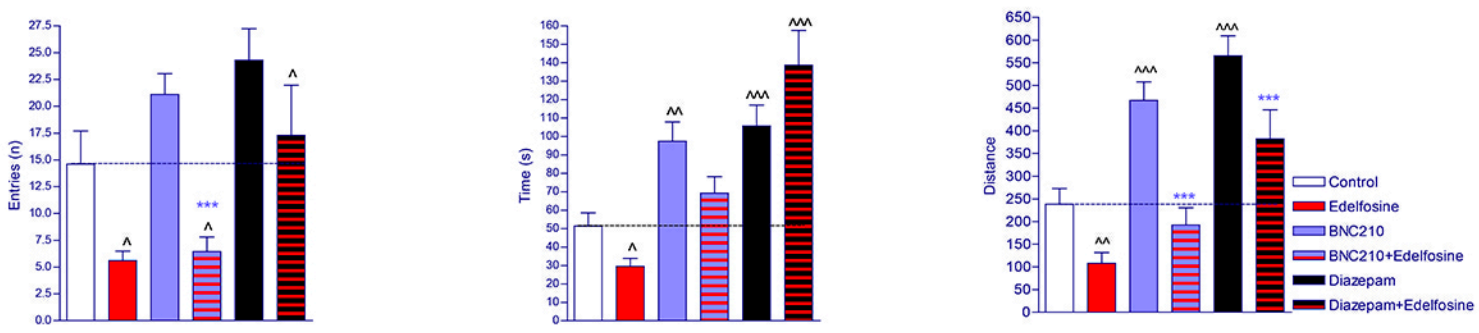


Figure 4: Edelfosine
Edelfosine alone (30 mg/kg; i.p.) produced anxiogenic behaviour in mice. BNC210 (10 mg/kg; p.o.) and Diazepam (1 mg/kg; p.o.) produced potent anxiolytic activity. When BNC210 was administered to mice pre-treated with Edelfosine, the anxiolytic effect of BNC210 was completely abolished and the mice remained very anxious. In mice pre-treated with Edelfosine, Diazepam was able to significantly reverse the anxiogenic response caused by Edelfosine with only a slight reduction in anxiolytic efficacy.

*p ≤ 0.05, **p ≤ 0.01, *** p ≤ 0.0001 significantly different from BNC210 or Diazepam treatment alone.
^ p ≤ 0.05, ^^ p ≤ 0.01, ^^^p ≤ 0.0001 significantly different from control group.
T-test; results expressed as mean ± SEM; n=10 mice. This work was funded by Bionomics Ltd.