BNC375, A Novel Positive Allosteric Modulator of the α7 Nicotinic Acetylcholine Receptor, Exhibits Cognitive Enhancement in Rodent Behavioural Models

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Cholinergic neurotransmission has long been implicated in processes of attention, cognition, and learning and memory. The α7 acetylcholine nicotinic receptor (α7 nAChR) is a promising drug target for diseases involving cognitive impairment such as Alzheimer's and schizophrenia. α7 nAChR positive allosteric modulators (α7 PAMs) are a novel class of drugs offering advantages over α7 full/partial agonists. In contrast to α7 agonists, α7 PAMs do not activate α7 nAChRs by themselves and do not desensitize them. Instead, α7 PAMs work via amplification of responses induced by intrinsic agonist which preserves spatio-temporal signalling patterns.

1. BNC375 is a potent and efficacious *α7* nAChR positive allosteric modulator.

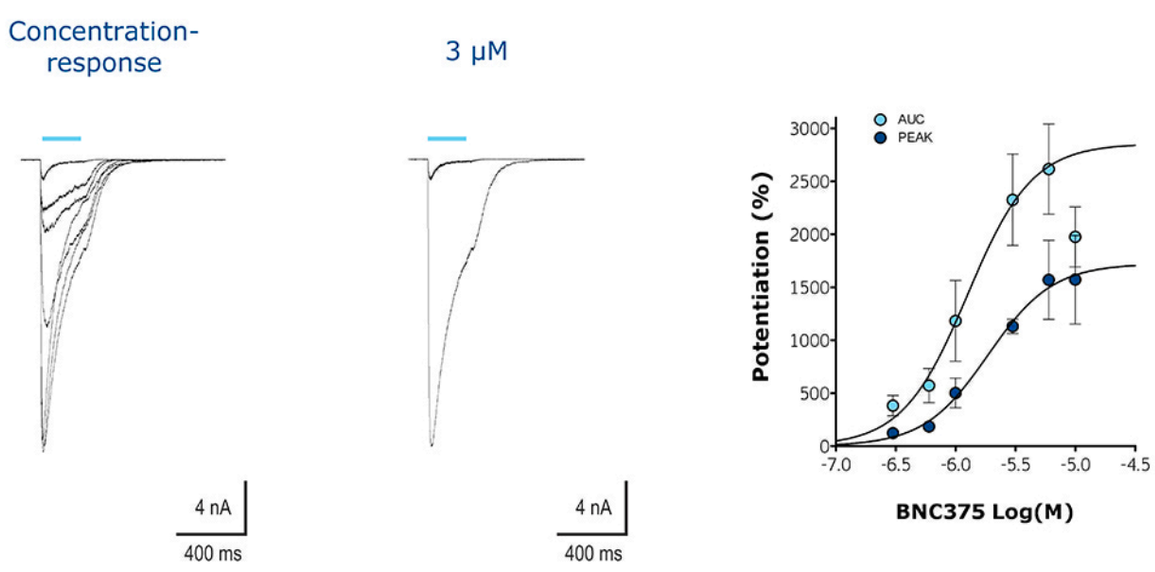

Figure 1. α7 nAChR-mediated current traces obtained from stably expressing GH4C1 cells on a Dynaflow® system. Concentration-response (*left*, 300nM-10 μM) and a representative trace of potentiation by BNC375 at 3 μM (*middle*). Application of ACh/PAM+Ach – 250ms (*blue bar*). *Right*, Concentration-response showing potentiation of peak current and area under curve (AUC) by BNC375. Fit parameters for peak potentiation: EC_{50} – 1.9 μM, n_H – 1.62, E_{max} – 1572%. AUC: EC_{50} – 1.3 μM, n_H – 1.6, E_{max} – 2616%.

2. Effects of BNC375 on ACh concentration-response curve

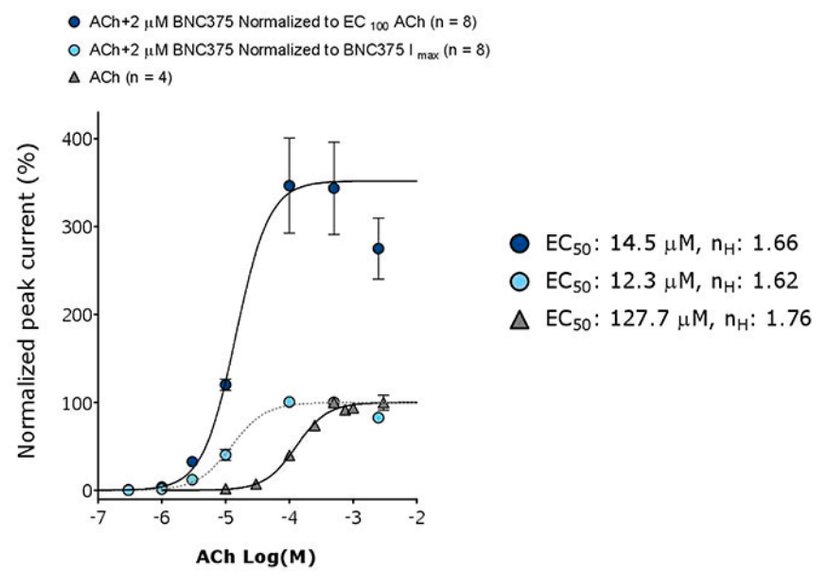

Figure 2. 2 μM BNC375 (~EC_{50}) shifts ACh concentration-responsen to the left and upward direction showing potentiation at both sub-threshold and saturating agonist concentrations (300 nM to 2.5 mM ACh).

3. Kinetics of *α7* nAChR-mediated currents and re-activation of desensitized receptors compared between BNC375 and a classical type II *α7* PAM PNU-120596

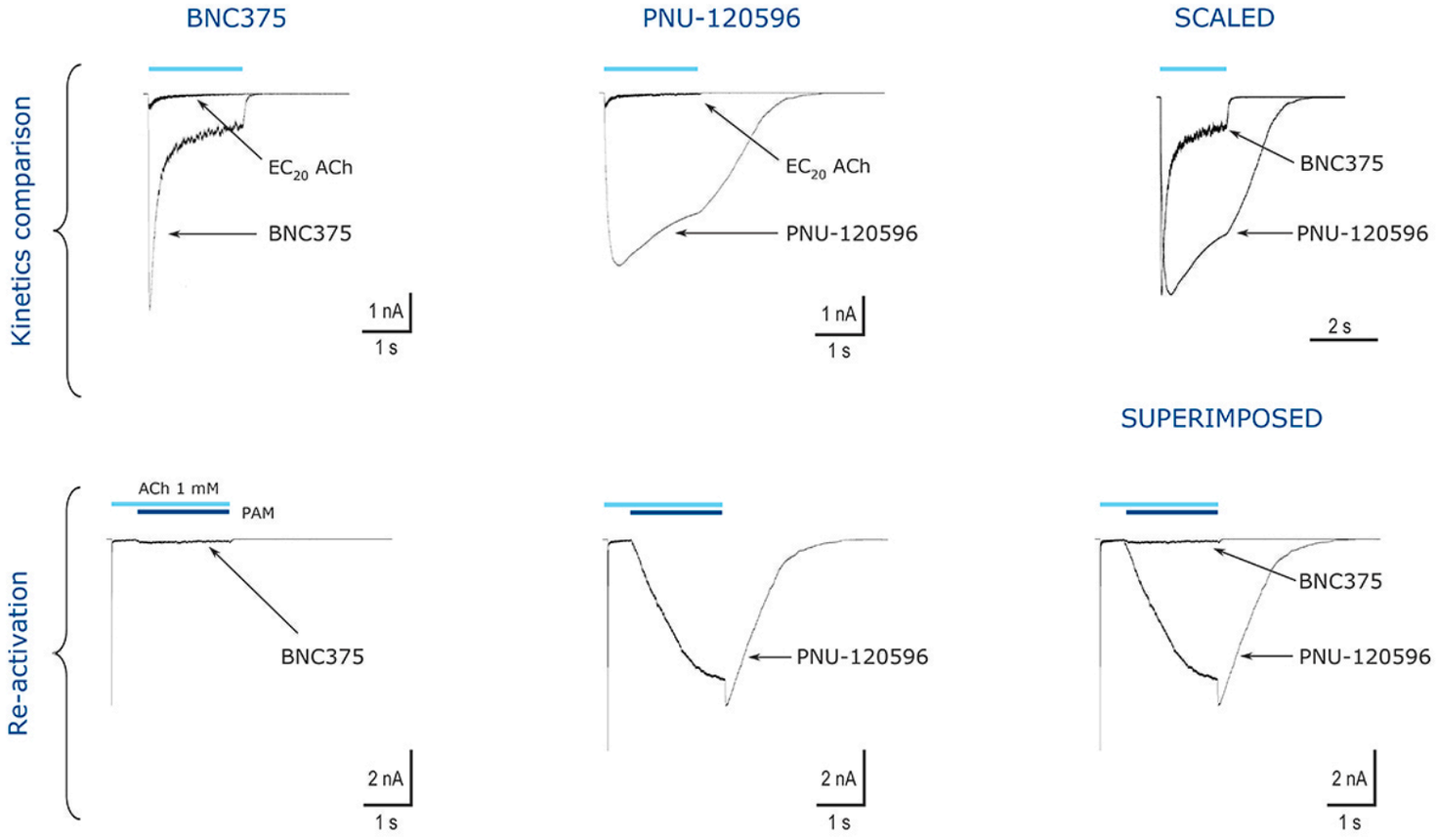

Figure 3. Kinetics of α7 nAChR-mediated currents after pre-incubation with either BNC375 or a 'classical' type II PAM PNU-120596 using 2 seconds application of ACh+PAM (*blue bar*) (*upper row*). Note that BNC375 has much more subtle effects on kinetic parameters of α7 currents as evidenced by the scaled traces (*upper right*), namely, no slowed activation and de-activation, and, especially, difference in kinetics of desensitization.

Effect of the compounds on re-activation of desensitized α7 nAChRs (*lower row*). α7 receptors were desensitized with a high ACh concentration (1 mM) after which either BNC375 or PNU-120596 was applied with ACh still present. Note that PNU-120596 re-activates desensitized receptors (induces the current) and BNC375 does not.

Overall, taking into account effects on desensitization and re-activation of α7 nAChRs, the kinetic profile of BNC375 is consistent with type I α7 nAChR PAMs.

4. BNC375 demonstrates in vivo cognition enhancing properties in rodent behavioural models

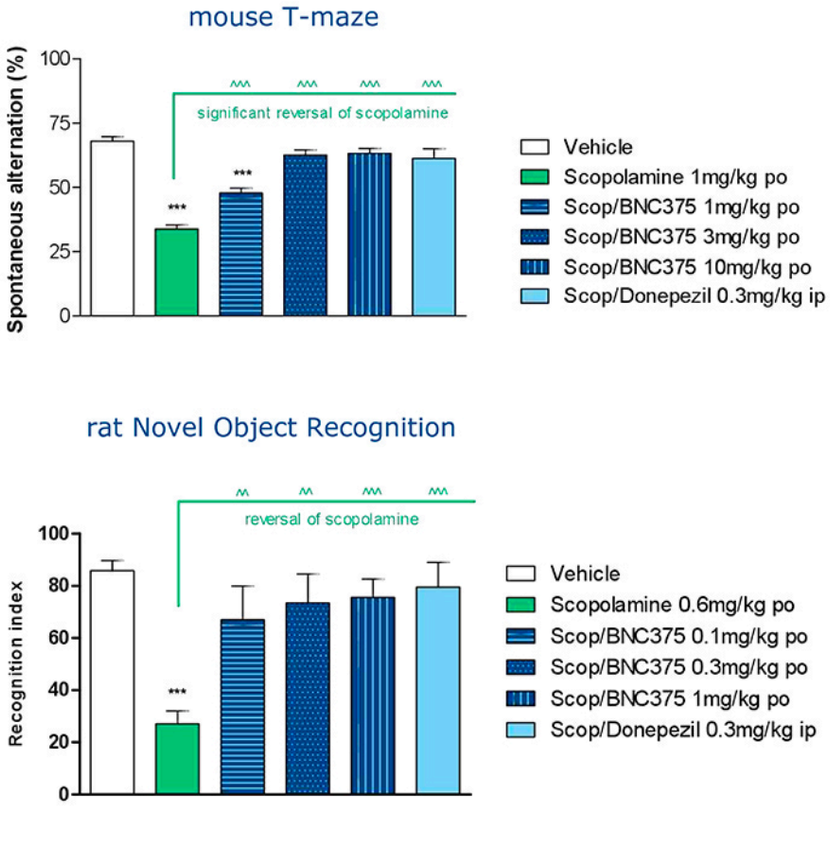

Figure 4. Behavioural tests in classical rodent cognition models show that BNC375 reverses scopolamine-induced impairment of spontaneous alteration score in T-maze Continuous Alternation Task (T-CAT) (*upper*, mice), or recognition index in the novel object recognition test (NOR) (*lower*, rats). See METHODS for details.

T-CAT is used for assessment of working memory and NOR for episodic memory. Note that therapeutic window for BNC375 is 0.1-10 mg/kg p.o. and that BNC375 matches performance of Donepezil.

METHODS

Electrophysiology:

GH4C1 cells stably expressing rat α7 nAChRs were patch-clamped in the recording chamber of 16-channel re-usable Dynaflow® ReSolve chips using EPC10 USB amplifier (HEKA Elektronik, Germany). Extracellular solution contained NaCl – 137 mM, KCl – 5 mM, $CaCl_2$ – 2.5 mM, $MgCl_2$ – 1 mM, HEPES – 10 mM, D-Glucose – 10 mM, pH – 7.4. Thin wall borosilicate glass electrodes (Harvard Apparatus) were pulled to a resistance of 2-4 MΩ when filled with intracellular solution (K^+-gluconate – 120 mM, KCl – 5 mM, HEPES – 10 mM, EGTA – 10 mM, $MgCl_2$ – 1 mM, ATP – 2 mM, pH – 7.2). Cells were held at -70 mV. Cells with series resistance below 15 MΩ were kept and 40% compensation was utilized routinely.

The recording protocol consisted of obtaining of two control ACh responses (EC_{20}, peak, 250 ms pulse) prior to 30 s pre-incubation with a tested compound (3 μM) followed by 250 ms co-application of 3 μM compound plus EC_{20} ACh. Dose-responses for selected compounds were obtained by a continuous compound application of increasing concentrations alternated with co-applications of compound plus EC_{20} ACh every 30 seconds.

Current amplitudes along with net charge carried (area under curve, AUC) were measured in Patchmaster software (HEKA Elektronik, Germany) and percentage of peak current and AUC potentiation by test compounds was calculated using the above mentioned formula. Dose-responses for selected compounds were fitted and plotted in Prism4/5 (GraphPad Software, Inc., CA).

In vivo characterisation was performed using the mouse T-maze Continuous Alternation Task (T-CAT) (Spowart-Manning & van der Staay, 2004) and the rat Novel Object Recognition (NOR) (Ennaceur & Delacour, 1988). Both models explored the ability of the compound to reverse a memory deficit induced by scopolamine. BNC375 was compared to vehicle and scopolamine treated animals for their reversal of the scopolamine–induced memory deficit.

T-MAZE: Scopolamine (1 mg/kg) and Donepezil (0.3 mg/kg) were administered to mice i.p., 20′ prior to testing. BNC375 was administered orally, 1 hour prior to testing. Percent of spontaneous alternation was measured over 14 free-choice trials or after 10 min had elapsed; n=10-20 mice.

NOR: Scopolamine (1 mg/kg and Donepezil (0.3 mg/kg) were administered to rats i.p., 20′ prior to testing. BNC375 was administered orally, 1 hour prior to testing. For each animal, the time taken to explore familiar object A (tA) and novel object B (tB) was recorded during a 10 minute period and the recognition index (RI) determined using the formula RI = tB/(tA + tB) x 100; n=12-22 rats

Statistical analyses: were performed using the student's t-test. P values indicating significant difference to scopolamine treatment: ^p≤0.05, ^^ p ≤ 0.01, ^^^ p ≤ 0.0001. P values representing significant difference to vehicle treatment only: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.0001

CONCLUSIONS

- BNC375 is an α7 nAChR PAM
- Effective across a wide range of agonist concentrations (from subthreshold to saturating).
- Favourable type I-like kinetics mainly affecting the peak α7 current with little effect on current desensitization.
- Demonstrates efficacy in animal models of episodic and working memory with a broad therapeutic window (100 fold).
- Matches performance of Donepezil.
- BNC375 is Bionomics' clinical candidate for Alzheimer's disease entering into IND enabling studies.

(Also see BNO announcement from 14/12/2013)
http://www.bionomics.com.au/siteFiles/files/news/Announcements_497.pdf
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