In vitro evaluation of AUT00206, a novel and selective Kv3 channel modulator for the treatment of schizophrenia

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Abstract

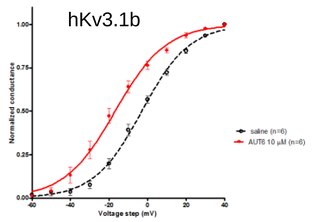
Background: Accumulating evidence supports a central role of fast spiking GABAergic interneurons in the pathophysiology of schizophrenia. Dysfunction of these interneurons, which is associated with reductions in the calcium binding protein, parvalbumin (PV) leads to disinhibition of cortical circuitry, dysregulation of gamma oscillations, and is thought to contribute to cognitive deficits observed in patients with schizophrenia (Lewis et al. TiNS 2012; 35: 57–67). Voltage gated, Kv3.1 potassium channels are selectively expressed by PV interneurons in cortical circuits, where they permit rapid and accurate firing necessary to synchronise the coordinated firing of pyramidal principle neurons at gamma frequencies. Kv3.1 channels are found to be reduced in un-medicated schizophrenia patients (Yanagi et al. Mol Psychiatry 2014; 19: 573-579). Modulation of this channel may therefore provide a means to restore PV interneuron function in schizophrenia patients, and improve cognitive and perhaps, negative symptoms, an unmet clinical need. This study describes *in vitro* assessment of the pharmacology of a novel, first-in-class Kv3 channel modulator, AUT00206, which has proven effective at improving cognitive and social behavioural deficits in a sub-chronic PCP (scPCP) model of schizophrenia symptoms (see also Neill et al., this meeting), and is currently in early clinical development for the treatment of schizophrenia (see also Hutchison et al. this meeting). The present study further explores the compound's ability to enhance gamma frequency cortical network synchrony in an animal model for schizophrenia pathology, and in *in vitro* brain slices obtained from human patients.

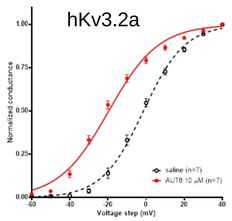
Methods

In vitro pharmacology studies were conducted using mammalian cell lines stably expressing human Kv3.1 and Kv3.2 channels. Standard patch-clamp recording techniques were used to determine the effects of AUT00206 on Kv3.1 and Kv3.2 channel function. For native tissue studies, cohorts of adult female Lister-Hooded (LH) rats received phencyclidine (2 mg/kg, scPCP) or saline i.p. for 7 days, followed by 6 weeks washout. This procedure has been shown to produce enduring cognitive and social behavioural deficits associated with reduced PV in frontal cortex and hippocampus (Neill et al. 2010. Pharmacol & Ther. 2010; 128(3): 419-432). Rats were then tested to confirm cognitive deficits using a novel object recognition task. Prefrontal cortical slices of the prelimbic and infralimbic regions were prepared from these rats and efficacy of AUT00206 to modulate kainate/carbachol-induced fast (20-80 Hz) network oscillations was examined. Human frontal or temporal neocortex slices were obtained from patients undergoing brain surgery for removal of a tumour or epilepsy focus. Effects of AUT00206 on gamma oscillations induced by kainate/carbachol in the presence or absence of PCP was investigated in these slices.

AUT00206 Modulates Human and Rat Kv3 Channels

AUT00206 produced a concentration-dependent leftward shift of the voltage-dependence of activation of hKv3.1b and hKv3.2 channels:

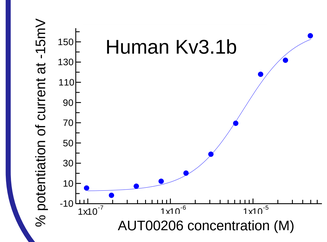


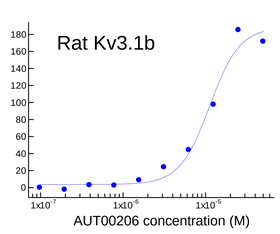


AUT00206	hKv3.1b	hKv3.2a
10 µM	- 7.1 mV	- 11.2 mV
30 µM	- 15.9 mV	- 29.2 mV

Voltage shifts are corrected for the effect of vehicle (0.1% DMSO)

AUT00206 produced a concentration-dependent potentiation of currents mediated by human and rat Kv3.1b channels:



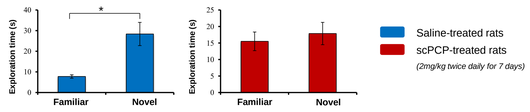


AUT00206	hKv3.1b	rKv3.1b
EC50	8 µM	9 µM

Graphs show example curves from individual experiments; table data are mean values for n=4 experiments.

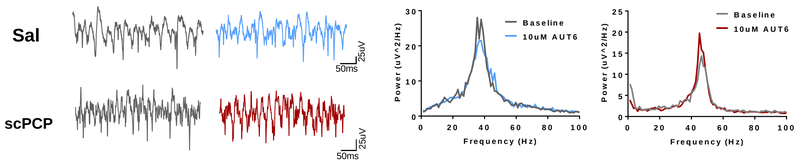
AUT00206 enhances gamma frequency oscillations in cortical slices from scPCP rats with cognitive deficits

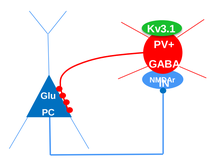
scPCP rats show cognitive deficits in the Novel Object Recognition task



Gamma oscillations are entrained by PV+ interneurons that express Kv3.1 channels

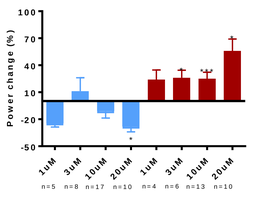
AUT00206 (10 µM) augments gamma oscillations in prefrontal slices from scPCP, but not saline treated rats





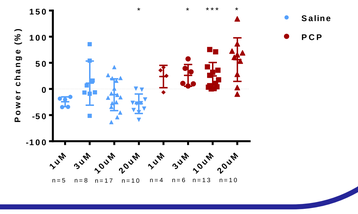
The effects of AUT00206 (10 µM) are most evident in prelimbic cortex and are concentration dependent. Higher concentration of AUT00206 produced a significant reduction in gamma power in slices from saline-treated rats

Group data (mean ± sem)

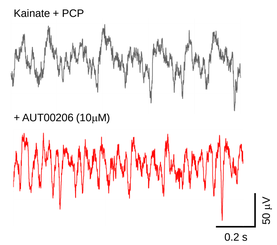


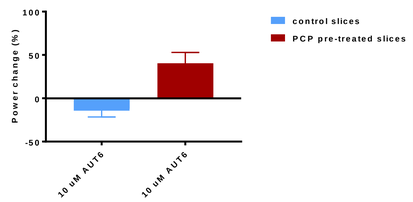
* - *** P<0.05 – 0.001. Wilcoxon test on raw values of power (pre-drug baseline vs AUT00206 at 60 min)

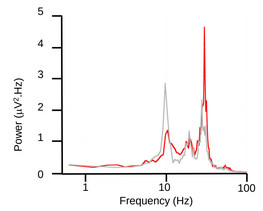
Individual slice data (mean ± SD)



AUT00206 enhances gamma frequency oscillations in cortical slices from human subjects



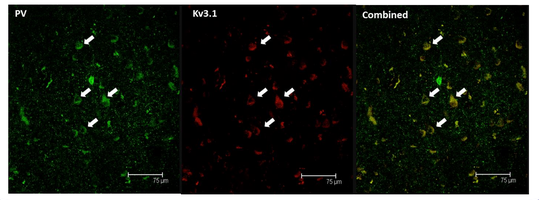




AUT00206 (10 µM) restored and enhanced gamma-frequency oscillations in human cortical slices following PCP, but not saline treatment *in vitro.*

Data shown in each group are from 3 slices from 2 human subjects.

Immunohistochemistry confirmed the co-localisation of Kv3.1 channels on PV+ interneurons:



Summary and Conclusions

AUT00206 positively modulates recombinant human Kv3.1 and Kv3.2-mediated currents, associated with a leftward shift of channel activation. The compound has a weaker effect on Kv3.3 and Kv3.4 channels, and little or no effect on other ion channels tested, as well as a wide range of other receptors, enzymes and transporters (data not shown). In native tissue experiments, AUT00206 enhanced gamma oscillations in cortical slices from scPCP treated, but not normal rats, and slices acutely treated with PCP from humans. These effects are consistent with the modulation of Kv3 channels on PV interneurons by AUT00206, and suggest potential of the drug to provide a novel approach for improving cognitive function in patients with schizophrenia.

Disclosures

Sources of financial sponsorship: This work is supported by Innovate UK and Autifony Therapeutics Limited.

Declaration of interests: CL, NP, and GA are employees and shareholders in Autifony; MC, FL and TM have received expenses to attend conferences and fees for lecturing, consulting and attending advisory boards from different pharmaceutical companies.