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Reply to Greig et al. *"Amyloid precursor protein synthesis inhibitors for Alzheimer's disease treatment"*

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We appreciate the interest of Dr. Greig and colleagues in our study, which identified 2-PMAP as a novel translational modulator of the APP expression[1]. As noted in their letter, we used CHO APP_{751SW} cells to compare the potency of 2-PMAP, with that of phenserine, whose effect on lowering APP expression was previously published by Greig's group[2,3]. In this comparison, both compounds showed dose-dependent effect on the APP expression and Aβ secretion with 2-PMAP inhibiting $Aβ_{\mathbf{x}\text{-}40}$ and $Aβ_{\mathbf{x}\text{-}42}$ production with a minimum effective concentration (MEC) of 0.1μM and 0.5μM, respectively; while phenserine showed a MEC of 5μM and 25μM, respectively. In their commentary, Dr. Greig furnished previously unpublished data about his group's negative experience with testing phenserine in CHO clones transfected with human APP, although pointedly he does not state the source, characteristics or validity of his clones or indeed whether they are the same clones that we have used. He attributes the lack of phenserine effect to the absence of or incomplete 5′ untranslated region (UTR) of APP mRNA in the construct they used as this sequence plays a key regulatory role in translational efficiency of APP mRNA into protein[4,5]. We used CHO APP_{751SW} line developed by cloning in the complete APP cDNA, the transcription of which produces APP mRNA containing the open reading frame flanked by 3′ UTR and 5′ UTR regions[6–8]. Furthermore, our demonstration that phenserine lowers the APP level and Aβ secretion in CHO APP_{751SW} cells indirectly confirms the presence of a functional APP 5′ UTR sequence in this model.

One difference between our experimental protocol and that of Dr. Greig's group was that, we used free base versions of 2-PMAP and phenserine, which were first dissolved in dimethyl sulfoxide (DMSO) and then added to the cell culture media, while Dr. Greig and colleagues used phenserine tartrate, which they directly dissolved in the media[3]. We would

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Potential Conflicts of Interests:

A.A.A.: nothing to report; J.E.P.: married to M.J.S.; M.J.S.: paid educational presentations, Forest Pharmaceuticals; consultancy, Phillips North America; co-inventor on US Patent No. 8,658,677 "Pyridil-2-methylamino compounds, composition and uses thereof", which is related to the work described in this article. This patent is licensed by NYU to Aria Neurosciences Inc. and M.J.S. is entitled to a share in the resulting licensing proceeds payable to NYU. M.J.S. has no ownership interests in, outside position with, or other contractual relationship with Aria Neurosciences Inc.

like to stress that besides phenserine, 2-PMAP also has limited water solubility what requires solvents like DMSO to prepare the initial stock solution for cell culture experiments. We performed our experiments carefully, and we did not appreciate the formation of any precipitates in the conditioned media after adding DMSO stock of either compound. Therefore, we are not convinced that our approach to solubilize phenserine limited its bioavailability, and if so, it could potentially do the same for 2-PMAP.

Another issue raised by Dr. Greig concerns the prediction of prospective *in vivo* efficacy of the CNS therapeutics based on their cell culture testing data. Dr. Greig stated accurately that the concentrations of phenserine required to affect Aβ reduction in the CHO APP_{751SW} cells are unachievable in the brain. In their studies, they found SH-SY-5Y neuroblastoma line to be far more sensitive to show the effect of phenserine on the APP expression level and reported the IC_{50} of phenserine in this line around 1μM[3]. However, they also reported that 50μM concentration of phenserine is required in SK-N-SH neuroblastoma line to effect significant reduction in the APP level[2]. Effects of phenserine on Aβ secretion in SH-SY-5Y cells has not been reported, while in SK-N-SH line, a 50μM concentration was required to significantly lower Aβ production[2,3]. It is uncertain why one neuroblastoma line is more favorable than the other; however given illustrated differences in phenserine potency among closely related neuroblastoma lines, it is difficult to agree with Dr. Greig's comment that our data on phenserine testing in CHO APP_{751SW} cells provide misleading characterization of this compound. Furthermore, recently Roger's laboratory using a pIRES-APP-5′ UTR construct identified several highly potent APP translational inhibitors targeting the 5′ UTR of APP mRNA sequence with IC_{50} around 0.1μM[4]. Since phenserine also possesses 5′ UTR conferred activity, it was used for direct comparison in this model and showed IC_{50} of 5μM. Taken together, cell lines may constitute effective tools for screening and direct comparisons of compounds, but have limited utility for predicting their actual effectiveness *in vivo*. Therefore, both 2-PMAP and phenserine were tested in subacute animal experiments and both demonstrated the ability to lower brain APP and Aβ levels[1,3].

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