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Letter to Editor

Title:

NMDA Receptor Potentiation and Severe Acute Respiratory Syndrome Treatment

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Glutamate is the major neurotransmitter of the central nervous system and has diverse roles in the periphery. The N-methyl-D-aspartate (NMDA) receptor is a major subtype of glutamate receptors, which are predominantly expressed throughout the nervous system and in all vital organs in the human body (Kim et al., 2014). Functional NMDA receptors are hetero-tetramers composed of two identical glycine binding GluN1 subunits and two identical or different glutamate binding GluN2 subunits, of which there are four subtypes GluN2A-D (Paoletti, Bellone & Zhou, 2013; Traynelis et al., 2010).

Expression of NMDA receptors in lungs: The human proteome project identified abundant expression of NMDA receptor subunits in various organs outside the central nervous system including lungs, esophagus, and T-helper cells (Kim et al., 2014). This finding corroborates a large number of previous reports on the extra neuronal expression of NMDA receptors in various animals (Deng, Valdivielso, Munger, Blantz & Thomson, 2002; Dickman, Youssef, Mathew & Said, 2004; Erdo, 1991; Genever et al., 1999; Gonzalez-Cadavid, Ryndin, Vernet, Magee & Rajfer, 2000; Inagaki et al., 1995; Kim et al., 2014; Krizbai et al., 1998; Leung et al., 2002; Li et al., 2018). NMDA, when applied to perfused tracheal segments of guinea pigs, increased resting muscle tone and enhanced the contractile response to acetylcholine. In whole guinea-pig lungs, when administrated through the trachea, NMDA increased airway perfusion pressure and this increase was totally abolished by NMDA receptor channel blocker MK-801. In addition, recent studies support critical role of endogenous glutamate on NMDA receptor function during acute lung injury and airway inflammation (Dickman, Youssef, Mathew & Said, 2004; Said, Dey & Dickman, 2001; Said & Dickman, 2000; Said, Pakbaz, Berisha & Raza, 2000). An NMDA receptor

blocker could impair fetal rat lung development (Liao et al., 2016; Wang et al., 2016). GluN1/2A and 2B subtypes were not identified in the lung cells, whereas GluN1/2C subtype was found to be expressed in peripheral and mid-lung samples. GluN1/2D subunit was predominantly expressed in the peripheral, gas-exchange zone of lung and in alveolar macrophages, and this expression was upregulated in lungs treated with NMDA (Dickman, Youssef, Mathew & Said, 2004).

Kinetics of NMDA receptor subtypes: Glutamate, with concurrent binding of the co-agonist D-serine or glycine, activates NMDA receptor that non-selectively conduct ions across the cells at depolarizing membrane potential which unbinds the otherwise blocking Mg^{2+} ions. NMDA receptor mediated transport of calcium and sodium ions into the cytoplasm is essential for excitatory cellular events that results in human airway smooth muscle contraction (Anaparti et al., 2015). Each non-GluN1 subunit confers distinct spatiotemporal expression and biophysical properties that result in varying agonist affinity, magnesium sensitivity, ion conductance, activation kinetics, open probability, mean open time, cellular localization and downstream signaling mechanisms (Traynelis et al., 2010). In general, diheteromeric NMDA receptors (GluN1/2) exhibit deactivation times that span about 50-fold range, with the following order (from fastest to slowest): NR2A < 2C < 2B << 2D(Cull-Candy & Leszkiewicz, 2004). GluN1/2A subunit containing NMDA receptor deactivation time constant is about ~50ms, GluN1/2B ~400ms, GluN1/2C ~290ms and GluN1/2D is >1second. Thus, activation of GluN1/2D subtype of NMDA receptors can conduct a large amount of calcium and sodium ions into the cells in which they are expressed. Therefore, potentiating NMDA receptor subunits that are predominantly expressed in the lungs should be helpful to improve the function of cells that are failing to contract.

NMDA receptor potentiators: In recent years, a variety of NMDA receptor potentiators have been identified, and they exhibit distinct subunit selectivity and mechanism of action (Costa et al., 2010; Monaghan, Irvine, Costa, Fang & Jane, 2012; Mullasseril et al., 2010; Perszyk et al., 2018; Perszyk et al., 2020). These compounds have been largely studied for their activities in neuronal NMDA receptor populations, with the aim of developing treatments for neurological and psychiatric disorders. However, these compounds and their analogs might have therapeutic potential for non-CNS disorders but this has not been explored. Since GluN1/2C&D subunits of NMDA receptors are predominantly expressed in the lung epithelial cells and macrophages, and being the slowest channel (among other glutamate receptors) to deactivate, these receptors can conduct large amount of calcium and sodium ions into the cells and trigger cellular contractions. Thus, GluN1/2C and/or 2D potentiators could be useful to stimulate the lung epithelial and smooth muscle cells during injuries or acute infectious conditions that suppress normal function of these cell types.

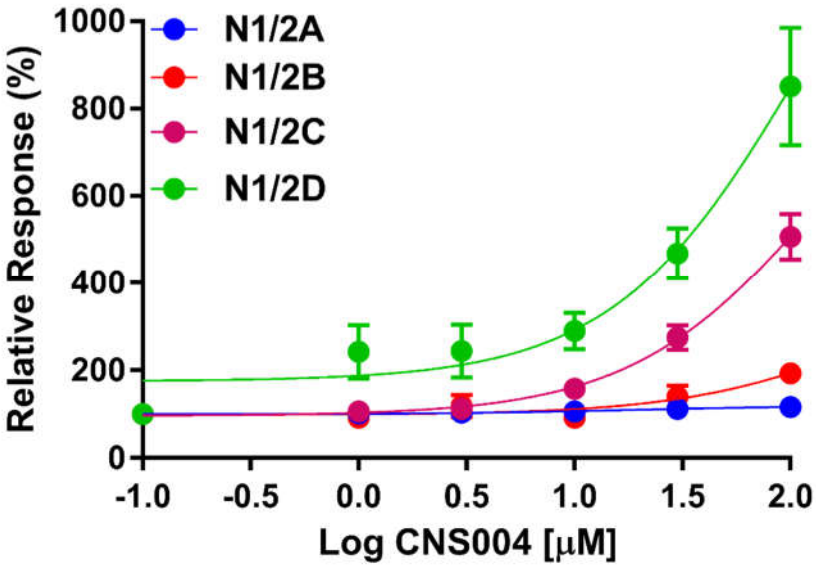
Coincidence or scientific connection between NMDA receptor drugs and antiviral properties: One of the clinically used antiviral agents, amantadine, is a potent NMDA receptor antagonist (Blanpied, Clarke & Johnson, 2005). This drug is also an FDA approved drug of choice (brand name: Gocovri) for the treatment of dyskinesia in patients with Parkinson's disease. Another drug, memantine (brand name Namenda), an analog of amantadine, is one of two FDA approved clinically used drugs for moderate to severe symptoms of Alzheimer's disease. Since both amantadine and memantine are chemically similar adamantine derivatives, memantine also exerts antiviral effects as previously reported (Brison, Jacomy, Desforges & Talbot, 2014). Memantine is known to bind at a highly conserved extracellular vestibule of NMDA receptor channel by displacing magnesium ions that block NMDA receptors at resting membrane potential. However, existence of nucleic acid sequences that would translate to NMDA receptor channel or similar ionotropic glutamate receptor proteins are not identified in any known viral genome. Therefore, adamantine derivatives might affect the expression or function of host cell biomolecules including proteins involved in viral particle internalization, replication, assembly and

release of matured virion. While these mechanisms could help treat viral diseases, theoretically, NMDA receptor blockers might not necessarily improve the symptoms of airway dysfunction. Conversely, a potentiator could possibly improve the airway function.

A novel NMDA receptor potentiator with antiviral and immunomodulatory effects: Through *in-silico* search, we have identified a compound (coded as CNS004) that modulates NMDA receptor function based on GluN2 subunit composition. CNS004 selectively potentiated GluN1/2D receptor currents up to 8-fold, when activated by 100 μM glycine and 0.3μM glutamate, and no effect on GluN1/2A and 2B receptors (Figure.1). This compound has variety of other biological activities as reported by the National Center for Advancing Translational Sciences (NCATS). Following are four different activities (and Pubchem bioassay ID) including an anti-viral activity against influenza-A virus non-structural protein-1 (2326); anti-malarial, inhibitor of apical membrane antigen-1 of plasmodium falciparum (720542); antiprotozoal, inhibitor of fructose 1,6 bisphosphate aldolase from Giardia Lamblia (2451) and inhibition of nuclear receptor ROR-gamma in the immune cells (2551 & 2546). Chemical structure of CNS004 and more details on these activities can be obtained from Pubchem with compound CID# 3794169.

A multipronged approach to treat viral injections: An NMDA receptor potentiator with antiviral properties could serve as novel treatment strategy for severe acute respiratory syndrome (SARS). Potentiating the activity of NMDA receptors expressed in the respiratory tract will increase calcium ion influx and promote downstream signaling mechanisms associated with cellular contractions that are possibly impaired or downregulated during SARS. Pharmacological effects generated by triggering CNS NMDA receptor function, coupled with concurrent potentiation of NMDA receptors expressed in the respiratory tract, could produce a synergetic effect in improving airway smooth muscle contractions. Further, an anti-viral activity combined with immunomodulatory effect of CNS004 may result in Covid-19 symptom improvements. We are currently working to determine the molecular mechanisms of this compound using *in-vitro* and *in-vivo* models of SARS-CoV2. Future research should be directed towards identification of novel NMDA receptor modulators that serve the purpose of this multipronged approach for the treatment of SARS.

Figure.1: Averaged and normalized CNS004 dose-response curves of NMDA receptor subunits. GluN1 and GluN2 subunit cRNA were injected into xenopus oocytes 48-72hrs before performing two electrode voltage clamp electrophysiology assay in -60mv holding potential. 100μM glycine and 0.3μM glutamate were used as agonist to activate the receptor while performing CNS004 dose response curves. Agonist induced whole cell currents were normalized to 100% to calculate potentiating effect of CNS004. A manuscript presenting original data of this figure is currently under review in the British Journal of Pharmacology (ID: 2020-BJP-1810-RP).


N1/2A
N1/2B
N1/2C
N1/2D
Relative Response (%)
1000
800
600
400
200
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log CNS004 [μM]

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