Effects Of Ifenprodil On Di- And Tri-heteromeric Channel Properties Of NMDA Receptor Channels In Dopaminergic Neurons Of Neonatal Rat Substantia Nigra

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NMDA receptors are ubiquitously expressed in the central nervous system and are generally composed of two glycine-binding NR1 subunits and two glutamate binding NR2 subunits. While many receptors are diheteromers of NR1 and a single type of NR2 subunit, there is evidence for triheteromeric NR1/NR2B/NR2D receptors in the midbrain (Dunah et al., 1998; Jones & Gibb, 2005) and cerebellum (Brickley et al., 2003). We have used patch-clamp single channel and whole-cell recordings to quantify the properties of NMDA receptors in SNc dopaminergic neurons in 300 µm thick midbrain slices from 7 day old rats.

Responses to a high concentration (200 µM) of NMDA with 10 µM glycine in the presence of TTX (100 nM) were recorded before and after the application of the NR2B-selective antagonist, ifenprodil. At -60 mV, 10 µM ifenprodil gives 55.9±2.7% blockade of NMDA receptor-mediated whole-cell currents. Using outside-out patch-clamp recordings the concentration-inhibition relationship for the action of ifenprodil on single channel charge transfer was fitted with a single-component Hill equation with IC₅₀ = 0.39 µM. As 10 µM ifenprodil would be expected to block diheteromeric NR1/NR2B receptors by around 90%, these data show that other NMDA receptors are contributing to the NMDA receptor current, or suggest that the receptors are triheteromers as N-terminal domain ligands like ifenprodil have reduced inhibitory efficacy at triheteromeric NMDA receptors (Hatton & Paoletti, 2005).

Analysis of single channel amplitude distributions with low concentration (100 nM) NMDA before and after 1 µM ifenprodil demonstrated that ifenprodil reduced the NMDA channel open probability (P_open) from 0.0102±0.0005 to 0.0077±0.0006 (n=3 patches, P<0.05, Students’ t-test). The total number of channel openings in the record were not affected by ifenprodil (control 639.1±9.2, ifenprodil 561.5±17.5; P>0.05, n=3), but 1 µM ifenprodil caused a 38.3% reduction in the mean open time of 50 pS openings and a 39.4% reduction in the mean open time of 40 pS openings and no significant effects on 17 pS openings. As 17 pS openings are characteristic of NR2D subunit-containing NMDA receptors, these results suggest that some receptors in the P7 substantia nigra are diheteromeric NR2D receptors.

Analysis of direct transitions between single channel current levels shows that 8.6% of direct transitions are between 50 pS and 17 pS conductance levels suggesting that both NR2B and NR2D subunits are within the same receptor molecule and contribute to formation of the channel pore.

These results suggest that extrasynaptic NMDA receptors in P7 rat SNc dopaminergic neurons are a mixture of triheteromeric NR1/NR2B/NR2D and diheteromeric NR1/NR2D receptors.