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Research paper

Nitric oxide in glutamate-induced compound action potential threshold shifts

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Abstract

Objective: Investigate the role of NO as a neurotransmitter in the gerbil cochlea and the effects of (7-NI) on compound action potential (CAP) threshold elevations induced by L-glutamate, an agonist at the NMDA glutamate receptor subtype, to further elucidate the role of NO in cochlear excitotoxicity.
Method: In anesthetized gerbils, CAP thresholds were recorded before and after cochlear perfusions with a control solution of artificial perilymph (APS) and a test solution of L-glutamate (GA) in three experimental groups.
Results: The control group showed no CAP threshold elevations ($p < 0.05$) when APS was perfused after systemic pre-treatment with 7-NI. GA perfusion alone caused significant elevation ($p < 0.05$) of the mean cochlear CAP threshold (25 dB SPL ± 5.8 dB to 78 dB SPL ± 19.5 dB). The CAP threshold elevation was prevented ($p < 0.05$) when the animals were pretreated with 7-NI before GA perfusion (24 dB SPL ± 4.2 dB to 27 dB SPL ± 6.7 dB).
Conclusion: NO mediates excitotoxicity when the cochlea is perfused with L-glutamate.

Keywords: Cochlea; Excitatory amino acids; Glutamate; Nitric oxide; 7-Nitroindazole; Excitotoxicity

1. Introduction

The excitatory amino acid (EAA) L-glutamate is thought to be the primary neurotransmitter acting at the synapse between cochlear hair cell and the afferent auditory nerve dendrites (Eybalin, 1993). Over-stimulation of glutamate receptors can lead to neuronal damage and disruption of cell electrophysiology (Garthwaite, 1991), a process referred to as excitotoxicity (Rothman and Olney, 1995). Part of the excitotoxic pathway involves massive intracellular influxes of calcium (Ca^{+2}) ions, resulting in the formation of calcium–calmodulin complexes and activation of neuronal nitric oxide synthase (nNOS), which catalyzes the formation of nitric oxide (NO) and peroxynitrate, ultimately leading to cell death. In the cochlea, excessive glutamate and aspartate have been shown to cause ototoxicity, as demonstrated by elevated cochlear compound action potential (CAP) thresholds (Bobbin and Thompson, 1978). The EAAs in the cochlea function primarily via three types of glutamate receptors: (1) *N*-methyl-D-aspartate (NMDA) ([Eybalin, 1993) which binds L-glutamate

Abbreviations: EAA, excitatory amino acid; Ca^{+2}, calcium ions; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; CAP, compound action potential; NMDA, *N*-methyl-D-aspartate; AMPA, quisqualate/alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; NOS, nitric oxide synthase; 7-NI, 7-nitroindazole; CNS, central nervous system; i.p., intraperitoneal; MAP, mean arterial blood pressure; APS, artificial perilymph solution; GA, glutamic acid/glutamate/L-glutamate; L-NAME, *l*-N^G-nitroarginine methyl ester; cGMP, cyclic guanosine monophosphate; $ONOO^-$, peroxynitrate anion; cAMP, cyclic adenosine monophosphate

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(2) kainate (Bledsoe et al., 1981; Jenison et al., 1986) (3) quisqualate/alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subtypes (Jenison et al., 1986). Activation of NMDA and kainate receptors are most commonly associated with excitotoxicity (Lefebvre et al., 1991).

Nitric oxide is enzymatically produced from arginine via nitric oxide synthase (NOS) and participates in numerous functions as a mediator of cellular responses within the central nervous system, including excitotoxicity (Dawson et al., 1991). NO specifically mediates EAA-induced cyclic GMP formation in post-synaptic structures (Garthwaite, 1991). Studies have shown that NOS is an active enzyme in cochlear spiral ganglion cells (Zdanski et al., 1994). Kainic acid, a conformationally restricted glutamate analog, is excitotoxic in the cochlea (Pujol et al., 1985) and elevates CAP thresholds (Bledsoe et al., 1981). Furthermore, 7-nitroindazole (7-NI), a competitive inhibitor specific for nNOS (Moore et al., 1993) significantly prevents CAP threshold elevations when administered systemically prior to kainic acid cochlear perfusion (Johnson et al., 1998). Based on these studies, we postulate that the function of NO in cochlea neuronal tissue is analogous to its function in the central nervous system (CNS), and that NO participates in EAA-induced excitotoxicity.

This study investigates the role of NO as a neurotransmitter in the gerbil cochlea and the effects of (7-NI) on CAP threshold elevations induced by L-glutamate, an agonist at the NMDA glutamate receptor subtype. The goal of this study was to further elucidate the role of NO in cochlear excitotoxicity.

2. Materials and methods

2.1. Surgical procedure

Mongolian gerbils (50–80 g) were anesthetized with an intraperitoneal (i.p.) injection of α-chloralose (100 mg/kg) and urethane (425 mg/kg) to provide surgical anesthesia, allow non-ventilator dependent oxygenation, and maintain mean arterial blood pressure (MAP) in the range of 80 ± 10 mmHg. A maintenance dose, 25% of the initial dose, was injected i.p. every 60 min to maintain constant blood levels.

Each animal was placed supine in a head holder apparatus and body temperature was maintained between 37° and 38 °C with a thermo-coupled, controlled heating pad. Surgery was performed only on animals in deep anesthesia, with regular breathing and adequate MAP. Anesthetics and non-specific inhibitors of NOS potentially affect blood pressure; therefore, blood pressure was monitored throughout the experiments using an intra-arterial cannula, which was placed in the carotid artery and connected to a blood pressure monitor (Cardiovascular Analyzer CVA-1, Buxco Electronics Inc., Sharon, CT, USA). The intra-arterial cannula was attached to a micro-infusion pump (Model A-99, Razel Scientific Instruments, Inc., Stamford, Connecticut) for continuous infusion of Krebs Ringer solution at a rate of 80 μl/min/kg. A ventral midline incision was made in the neck and a tracheostomy was performed. The right pinna was removed to expose the auditory meatus. The tympanic bulla, contralateral to the intra-carotid cannula, was opened carefully to expose the middle ear space while preserving the tympanic membrane and middle ear ossicles.

2.2. Cochlear perfusion

A circular hole, 50 μm in diameter, was drilled in the scala tympani at the first, basal-most turn of the cochlea for infusion, and a second hole, 40 μm in diameter, was similarly drilled at the apex to allow escape of the perfusate. This allowed perfusion of the entire scala tympani, from base to apex (Johnson et al., 1998). The position of the apical hole is critical, as the scala tympani and scala media are in closer approximation at the apex than at the base of the cochlea. A breach into the scala media would cause mixing of endolymph and perilymph, with subsequent disappearance of cochlear action potentials (Nuttall et al., 1977). The holes were created by carefully rotating a hand-held drill bit.

Glass micropipettes, to deliver the perfusate, were pulled on a vertical pipette puller (Model 700C, David Kopf Instruments). A 40 μm orifice was created by breaking the tip, and a small, silicone ball was fashioned a few micrometers above the tip (1–2577 conformal coating, Dow Corning, Midland, Michigan) to afford a tight seal. The pipette was connected via polyurethane tubing to a syringe and micro-infusion pump (Model #943, Harvard Apparatus Co., Millis, MA, USA). After evacuation of air bubbles, the pipette was lowered into the hole at the basal turn such that the silicone ball provided a tight seal between the pipette and the cochlea. All solutions were perfused at a rate 1.4 μl/min, a rate sufficiently slow enough to minimize mechanical interference on the cochlea. Perfusates were all 22 °C when infused to prevent confounding effects of cochlear cooling. Excess fluid was suctioned from the middle ear cavity with plastic tubing with care taken to avoid direct suction at and around both perfusion holes. A small amount of cotton was placed near, but not directly touching, the apical outflow hole to serve as a wick for excess perfusate. Excess perfusate was suctioned periodically from the cotton.

2.3. Solutions

The artificial perilymph solution (APS) was composed of 130 mM NaCl, 3.00 mM KCl, 1.25 mM KH_2PO_4, 10.0 mM glucose, 20.0 mM $NaHCO_3$, 1.20 mM $CaCl_2 \cdot H_2O$, and 1.30 mM $MgCl_2 \cdot 6H_2O$ dissolved in deionized water. Prior to each experiment, APS was filtered, maintained at 300 mOsm, and the pH was adjusted to 7.2–7.4 by bubbling 5% CO_2 through the solutions.

Glutamic acid (GA) (Sigma) was freshly dissolved in APS prior to each experimental perfusion. Test solutions