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Research Paper

Amino acid and acetylcholine chemistry in the central auditory system of young, middle-aged and old rats

Donald A. Godfrey*, Kejian Chen [1], Thomas R. O'Toole [2], Abdurrahman I.A.A. Mustapha

Department of Neurology and Division of Otolaryngology and Dentistry, Department of Surgery, University of Toledo College of Medicine, USA

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ABSTRACT

Older adults generally experience difficulties with hearing. Age-related changes in the chemistry of central auditory regions, especially the chemistry underlying synaptic transmission between neurons, may be of particular relevance for hearing changes. In this study, we used quantitative microchemical methods to map concentrations of amino acids, including the major neurotransmitters of the brain, in all the major central auditory structures of young (6 months), middle-aged (22 months), and old (33 months old) Fischer 344 x Brown Norway rats. In addition, some amino acid measurements were made for vestibular nuclei, and activities of choline acetyltransferase, the enzyme for acetylcholine synthesis, were mapped in the superior olive and auditory cortex. In old, as compared to young, rats, glutamate concentrations were lower throughout central auditory regions. Aspartate and glycine concentrations were significantly lower in many and GABA and taurine concentrations in some cochlear nucleus and superior olive regions. Glutamine concentrations and choline acetyltransferase activities were higher in most auditory cortex layers of old rats as compared to young. Where there were differences between young and old rats, amino acid concentrations in middle-aged rats often lay between those in young and old rats, suggesting gradual changes during adult life. The results suggest that hearing deficits in older adults may relate to decreases in excitatory (glutamate) as well as inhibitory (glycine and GABA) neurotransmitter amino acid functions. Chemical changes measured in aged rats often differed from changes measured after manipulations that directly damage the cochlea, suggesting that chemical changes during aging may not all be secondary to cochlear damage.

1. Introduction

The decline of hearing ability with age is a well-known occurrence that tends to isolate older adults from the rest of society. It includes loss of hearing and also difficulty perceiving spoken words within a noisy background. The underlying basis for these hearing deficits is not fully understood. Besides damage to the receptor cells of the inner ear during life, changes in the central auditory system appear to be involved (Willott, 1996; Frisina, 2001; Syka, 2002; Caspary et al., 2008; Richardson et al., 2013; Ouda et al., 2015; Stebbings et al., 2016). Since neural chemistry underlies neural function, age-related changes in the chemistry of central auditory regions, especially the chemistry underlying synaptic transmission between neurons, may be of particular relevance for hearing changes. Numerous studies have provided evidence of age-related changes in central auditory inhibitory neurotransmitter systems, especially those involving γ-aminobutyric acid (GABA) (Caspary et al., 2008, 2013; Richardson et al., 2013; Stebbings et al., 2016), whereas evidence regarding changes in excitatory neurotransmitter systems is more limited (Gold and Bajo, 2014). Quantitative assays of amino acid concentrations, including both inhibitory and excitatory neurotransmitters, in some whole auditory and vestibular regions were included among previous measurements for numerous brain regions of young and old Fischer 344 (F344) rats (Banay-Schwartz et al., 1989a,b,1990a,b) as well as adult and old people (Banay-Schwartz et al., 1992, 1993), and some age-related changes were reported. However, there is a lack of higher-resolution measurements of amino acid concentrations within subregions of central auditory structures.

* Corresponding author. Department of Neurology, Mail Stop 1195, 3000 Arlington Avenue, University of Toledo College of Medicine, Toledo, OH 43614, USA.
E-mail address: Donald.godfrey@utoledo.edu (D.A. Godfrey).
[1] Present address: Clinical Investigation Department, Naval Medical Center, San Diego, CA, USA.
[2] Present address: Department of Otolaryngology – Head and Neck Surgery, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA.

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Abbreviations

ALN	amygdaloid lateral nucleus
AudCtx, or AC	auditory cortex
AVCN	anteroventral cochlear nucleus
ChAT	choline acetyltransferase
CN	cochlear nucleus
DCN	dorsal cochlear nucleus
F344	Fischer 344
F344BN	Fischer 344 x Brown Norway
FNR	facial nerve root
GABA	γ-aminobutyric acid, or γ-aminobutyrate
GAD	glutamic acid decarboxylase
HPLC	high-performance liquid chromatography
IC	inferior colliculus
LSO	lateral superior olivary nucleus
LVN	lateral vestibular nucleus
MG	medial geniculate
MNTB	medial nucleus of the trapezoid body
MVN	medial vestibular nucleus
PVCN	posteroventral cochlear nucleus
SOC	superior olivary complex
SPO	superior paraolivary nucleus
VCN	ventral cochlear nucleus
VNC	vestibular nuclear complex
VNTB	ventral nucleus of the trapezoid body

In this study, we have combined microdissection procedures with high performance liquid chromatography (HPLC) to measure concentrations of amino acids, including the major excitatory and inhibitory neurotransmitters of the brain, in subregions of the major central auditory structures of young, middle-aged, and old Fischer 344 x Brown Norway (F344BN) rats. Results are presented for glutamate, the major excitatory neurotransmitter in mammalian brains, aspartate, a possible excitatory neurotransmitter closely related metabolically to glutamate (Lehninger, 1975; Dingledine and McBain, 1994), glycine and GABA, the major inhibitory neurotransmitters (DeLorey and Olsen, 1994), glutamine, an important precursor for synthesis of glutamate (Lehninger, 1975; Dingledine and McBain, 1994), taurine, which can act as an agonist at GABA and glycine receptors (Albrecht and Schousboe, 2005), serine, which can serve as a precursor for synthesis of glycine (Lehninger, 1975; Ross et al., 1995), and threonine and arginine, which are less closely related to neurotransmitter metabolism (Lehninger, 1975). Regions sampled include subregions of the cochlear nucleus (CN) - anteroventral cochlear nucleus (AVCN), posteroventral cochlear nucleus (PVCN), and dorsal cochlear nucleus (DCN); subregions of the superior olivary complex (SOC) - lateral superior olivary nucleus (LSO), medial nucleus of the trapezoid body (MNTB), superior paraolivary nucleus (SPO), and ventral nucleus of the trapezoid body (VNTB); subregions of the inferior colliculus (IC) – dorsal, ventral, and lateral; subregions of the medial geniculate (MG) – dorsal, ventral, medial, and lateral; and layers I–VI of the auditory cortex (AudCtx), particularly its primary part. In addition, the lateral nucleus of the amygdala (ALN) was sampled because of its connection with the MG and evidence for its involvement in emotional responses to auditory stimuli (LeDoux et al., 1990; Campeau and Davis, 1995; Masterton, 1997). From the same series of sections, for comparison to auditory regions, amino acid concentrations in young and old rats were compared in the first brain region of the other eighth-nerve sensory system, the vestibular nuclear complex (VNC), specifically in the lateral (LVN) and medial (MVN) vestibular nuclei. Lastly, the acetylcholine neurotransmitter system was checked in two auditory regions, by measuring choline acetyltransferase (ChAT) activity in the SOC and AudCtx.

Preliminary reports of some of the findings have been presented (Godfrey and Chen, 2009; O'Toole et al., 2009).

2. Materials and methods

The procedures used in this study are based on those described by Lowry and Passonneau (1972) and were the same as we have used for many of our previous studies (Godfrey and Matschinsky, 1976; Godfrey et al., 1988, 2000, 2008, 2012, 2013, 2014, 2015).

2.1. Animals

F344BN male rats of three ages were received from the National Institute on Aging. The six rats used in this study (Table 1) were deeply anesthetized with an overdose of sodium pentobarbital, decapitated 3 min later, and their brains isolated and frozen 4 min after decapitation in Fisherbrand Super Friendly Freeze-It (Fisher Scientific, Hampton, NH, USA) cooled to its freezing point with liquid nitrogen. Frozen brains were stored at −80 °C until sectioning.

Treatment of animals was approved by and in accordance with existing policies and regulations of the University of Toledo Health Science Campus Institutional Animal Care and Use Committee and the National Institutes of Health.

2.2. Isolation of tissue samples

Frozen coronal sections of each brain were cut 20 μm thick in a Hacker-Bright cryostat at −20 °C, including all central auditory regions from CN to AudCtx. For the more caudal part of the brain, through the CN, all sections were saved: every third for freeze drying, Nissl staining, or staining for cytochrome oxidase activity (rats B-F) or acetylcholinesterase activity (rat A). For the larger more rostral part of the brain, each group of 3 sections for freeze-drying and the two stains was alternated with 3 skipped sections. Sections for freeze drying were collected into aluminum racks (Lowry and Passonneau, 1972) kept on blocks of dry ice in the cryostat, and sections for staining were melt-mounted onto glass slides. Freeze drying was done overnight, with the aluminum racks inside a glass vacuum tube (Ace Glass Incorporated, Vineland, NJ, USA) held inside a freezer at −40 °C and attached through a dry ice trap to a vacuum pump (Lowry and Passonneau, 1972). The freeze-dried sections were stored within the aluminum racks inside the glass vacuum tubes under vacuum at −20 °C.

Freeze-dried sections were dissected into samples for assay on the Plexiglas stage of a Wild dissecting microscope at room temperature (19–22 °C), with relative humidity routinely below 50%. A

Table 1
F344BN rats used in the study.

Rat	Age (mo)	Weight (g)
A	6	360
B	6	379
C	22	536
D	22	560
E	33	548
F	33	476