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ABSTRACT

The present study describes significant, selective age-related changes in the rat inferior colliculus (IC) using two measures of GABA neurotransmission.

An area incorporating the central nucleus of the IC of the Fisher 344 rat was studied using an antibody against a GABA conjugate to immuno-label neurons and terminals in the IC. Using micropunches of the CIC the spontaneous and K+ evoked release of assorted of NTS was measured. Paired young adult (2-8 mo.) and aged (18-29 mo.) Fisher 344 rats were studied in order to compare numbers of GABA positive neurons. Computer morphometry was used to generate maps of the ventral lateral portion of the IC. Both labeled puncta and neurons were observed throughout the central nucleus of the IC. Immunostained terminals were observed on both large and small positive neurons as well as on negative neurons. A significant reduction (36%) in the number of GABA-positive neurons was observed in the aged animals when compared to their matched cohorts (145 neurons/mm2; $p < 0.01$). When the eight aged animals were compared to all 13 mature animals, a 47% decrease in the number of GABA immunoreactive neurons was observed (177 neurons/mm2 vs. 93 neurons/mm2; $p<0.005$). Preliminary observations from three mature-aged pairs of nissl stained sections suggest that a major proportion of the reduction in the total number of cells can be accounted for by the loss of GABA-positive neurons. The two-dimentional area of GABA-labeled IC neurons displaying nuclei and nucleoli was measured and no age related differences were observed (mean area 225 u2). Areas of the GABA-positive cells

displayed a broad distribution of neuronal sizes comparable to reported sizes for three subpopulations of IC stellate (multipolar) cells.(ref) At the upper size range of GABA-positive neurons fusiform profiles were also observed. age-related loss of GABA mediated inhibitory processes may occur.

INTRO

The inferior colliculus (IC) is an important auditory structure receiving ascending inputs from the cochlear nuclei (CN), superior olivary complex (SOC), nuclei of the lateral lemniscus (NLL), commisural projections, as well as indirect descending inputs from auditory cortex. A role for GABA as an inhibitory neurotransmitter in the IC has been suggested by a number of neurochemical and immunocytochemical studies. The IC has been found to contain high levels of glutamic acid decarboxylase (GAD) and GABA transaminase (GABA-T), enzymes responsible for the synthesis and degradation of GABA respectivelty, as well as GABA itself. (Fisher and Davies, '76; Tachibana and Kuriyama, '74; Adams and Wenthold, '79; Contreras and Bachelard, '79; Fisher and Davis, '76). These data are further supported by high immunocytochemical staining for GABA, GAD and GABA-T in the IC (Nagai et. al, '85; Moore and Moore, '87; Ottersen and Storm-Mathiessen, '84; Thompson et. al, '85). Neuropharmacological studies using iontophoretic application of GABA and GABA antagonists futher implicate GABA as an inhibitory neurotransmitter in the IC (Faingold et. al, '85, other refs?). Intracellular recordings from IC neurons in vivo and in vitro display IPSPs which can be blocked by picrotoxin, while bicuculline has been shown to block extracellularly recorded binaural

inhibition (Kuwada et. al, 1980; Moiseff, 1985; Smith, 1986; Faingold et. al, 1986).

Age related changes have been described in rat for auditory brainstem response (ABR) functions, glucose utilization, protein sysnthesis, GABA binding, and the ability to localize sound. (Simpson et al., 1985; London et al., 1981; Smith et al., 1980; Ingvar et al., 1985; Kendall et al., 1982; Brown, 1984; Harrison, 1981) Since GABA is an important neurotransmitter in the IC, the present study sought to examine loss of GABA and GABA neurons in this structure. Knowing the importance of GABA in the IC and the inhibitory role of this neurotransmitter, loss of GABA with age may be related to the loss of ability to process auditory information.

METHODS

Eight mature (2-8 mo., 250-300g) and eight aged (18-29 mo., 350-450g) Fisher 344 rats were paired off in order to process the animals simultaneously. Additionally 5 mature controls were processed. Animals were deeply anesthetized with Ketamine-HCl for transcardiac perfusions at a dose of 150 mg/Kg for the mature rats and 100 mg/Kg for the aged rats. A peristaltic pump was used to deliver 50 ml 0.1 M Sodium Cacadylate, pH 7.4 followed by 4% formaldehyde and 2% glutaraldehyde in 0.1 M sodium cacadylate, pH 7.4. Brains were carefully removed from the calvarium, blocked and post-fixed for 6-8 hrs. in 4.0% formaldehyde and 2% glutaraldehyde in 0.1 M sodium cacadylate, pH 7.4. Tissue blocks were then infiltrated with a graded series of phospate buffered sucrose (10%-30%) and stored until further processing.

A sliding microtome was used to cut twelve micron transverse sections into ice-cold 0.05 M Tris buffered saline (TBS). Free floating sections were processed in 13 ml gooch crucibles used as baskets to transfer sections between solutions in order to keep tissue handling to a minimum. Sections were washed in 0.05 M TBS, reacted with hydrogen peroxide (H2O2) in MeOH, pre-incubated in a blocking solution of normal goat serum (NGS), lysine, Triton-X in TBS, and then incubated with the primary antiserum diluted in a similar solution (GABA 1:500-1:1000) for up to 14 days at 4°celcius. This study utilized antiserum raised in rabbits against GABA congugated to bovine serum (Wenthold et al., 1985). Following labeling with the primary antibody, sections were incubated with goat anti-rabbit horseradish peroxidase (GAR-HRP) 1:100 in NGS and TBS. Reaction of the IgG-HRP with 3,3'-diaminobenzidine tetra-hydrochloride in the presence of H_2O_2 forms a dark brown, amorphous precipitate which is insoluble in water and alcohols (Granza, 1982). Sections were then mounted on gelatin subbed slides, dehydrated, and coverslipped. Three pairs of the brains were processed with alternate sections counterstained with cresyl violet.

A 50 mesh copper grid commonly used to support tissue for electron microscopy was carefully adhered with cyanoacrylic to the area of each slide containing the IC sections in order to deliniate a fixed area for quantitative analysis. Sections from the rat IC were compared to stereotaxic sections from a standard rat atlas (Paxinos and Watson, 1982) and matched descriptions by Rockel and Jones (1973) and Morest and Oliver, (1984). Only sections between

Bregma - 8.8 and Bregma - 9.3 (Paxinos and Watson, 1982) were chosen and only grid squares within the ventral lateral part of the central nucleus of the IC were studied. Sections were examined in a blinded paradigm under a Wild compound microscope fitted with a Dage 65 MKII video camera and coupled to a high resolution monochrome monitor at two magnifications (screen magnifications = 595X, and 5700X) with the latter allowing visualization of both nuclei and nucleoli. Immunoreactive profiles within predefined regions were identified, mapped, counted and stored for further analysis (Bioquant II, Digitizing Morphometry). The criteria for identifying immunostained neurons included the presence of a dark brown reaction product within cytoplasmic boundaries. Data were gathered from a minimum of fifteen sections from each animal, and a distribution pattern similar to Roberts et al. (1984) was generated.

RESULTS

A significant decrease (36%, $p<.01$) in the number of GABA-positive neurons in the ventral lateral portion of the central nucleus of the IC for eight aged rats was observed when compared with their paired mature cohorts. When an additional five mature controls were added to the paired mature rats and compared to the same eight aged animals a 47% ($p<.001$ check this!!!) decrease in the number of GABA neurons was observed. The pooled results presented in Table 1 are displayed graphically for each individual pair (N=8) of mature/aged animals in Figure 1 (bar graph).

Reduction in the number of GABA-labeled neurons per mm2 in the ventral lateral IC can be seen for both groups. Alternate sections

stained with cresyl violet in three mature/aged rat pairs were used to count all neurons in this area. Preliminary data from these animals suggest that a majority of the age-related loss of neurons in this area can be attributed to loss of GABA-positive neurons (Table 2).

When the two-dimensional area of GABA labeled IC neurons displaying nuclei and neucleoli was measured no significant age-related differences were observed (Table 1). Cross sectional areas of GABA-positive neurons displayed a broad distribution of neuronal sizes which were comparable to those reported in IC of the Sprague-Dawley rat described as three subpopulations of IC stellate (multipolar) cells ranging in size between 10-30um (Moore and Moore, 1987) and in the gerbil IC (Roberts and Ribak, 1987) using glutamic acid decarboxylase (GAD)-like immunoreactivity to label GABA neurons. At the upper size range of GABA-positive neurons some GABA-positve fusiform profiles were observed. (fig if needed)

DISCUSSION

The present study finds a significant loss in the number of GABA labeled cells in the IC of aged rats with respect to young adult rats. These findings support a number of other studies which display age-related changes in or relating to the IC, including: changes in protein sysnthesis, changes in glucose utilization, altered auditory brainstem response (ABR) functions, modifications in GABA receptor binding, and loss of the ability to localize sound. These changes may in turn relate to the loss of more peripheral auditory function often associated with age in both

human and animal subjects, but data in Fisher 344 rats and rats in general find relatively small changes at the periphery.

Studies in the Sprague-Dawley rat have demonstrated a significant decrease in protein synthesis in both the visual and auditory systems with age (Ingvar et. al, '85), while similar studies in both Sprague-Dawley and Fisher 344 rats show a decrease of 25% or more in the utilization of glucose in the IC of aged rat brains (Smith et. al, '80, London et. al, '81). Studies in the medial nucleus of the trapezoid body (MNTB, an inhibitory structure) indicate that the utilization of glucose in the auditory system is not related to the fuction of the structure whether it be excitatory of inhibitory; consequently, glucose utilization in GABA neurons is similar to neurons which are excitatory. (Masterton, ??) More recent studies using the 2-deoxyglucose method in the Fisher 344 rats in quiet as well as three different pure-tone stimulus conditions suggest that age-related metabolic changes occur in the IC in a regional and frequency dependent fashion (Clerci and Coleman, '87). Although it has been suggested that some of these losses may be due to degeneration of the peripheral auditory system and lower brainstem (refer to below), part of these losses are undoubtedly related to the reduction in the number of GABA positive cells in the IC, since a decrease in the number of cells would result in a decrease in all metabolic activity associated with these cells.

Binding studies in the Sprague-Dawley rat for receptors to GABA have shown an age-related decline in binding for most areas of the brain (Kendal et. al, 82). This decrease may be important

in the aging auditory system and IC in particular due to the importance of GABA as a neurotransmitter in the IC.

Auditory brainstem response (ABR) studies using both click and tone pip stimuli display changes in the senescent Fisher 344 rat which include prolonging of the predominant negative wave following wave IV (No) and increases in interpeak latencies independent of the increase in wave I latency (Simpson et al. 1985; Cooper et al., 1986). These data suggest that central transmission is altered in older animals, and since wave IV and notch N_o have been associated with the functioning of the IC, and the ABR is derived from the elctrical activity of the cells in that area and not from the inhibitory/excitatory functioning of individual cells (ref. if need one), the age-related changes in these ABR functions could be due to the loss of excitatory input, loss of response of SOC, or loss of GABA cells in the IC.

Directional hearing studies in SD rats show a loss in the ability to localize sound as aging progresses. (Brown, 1984: Harrison, 1981) Since the IC is an important structure in the processing of binaural information and GABA plays a known inhibitory role in the IC, (refs if we have them) the decrease in the number of GABA cells in the IC may be strongly related to the loss of the ability to localize sound with age, and consequently with age-related loss of hearing in general.

Studies of the auditory system in both human and animal models suggest a number of changes occur as this system ages. Morphological studies of the cochlea have described age-related loss of inner and outer hair cells, while age-related loss of

eighth nerve fibers has been reported to be as high as 20% in very old rats (Keithley and Feldman, '82; Hoeffding and Feldman, '87). Loss of hair cells could ultimately derease the input into the auditory brainstem which would alter functioning.

Results from studies of age-related neuronal losses in the human cochlear nuclei have been controversial, but when these data are compared to the findings reported in rodent, an age-related loss of coclear nucleus neurons most likely occurs. (Ref. if need one) Excellent studies carried out in rodents at the level of the superior olivary complex have described age-related morphologic changes (Casey and Feldman, '82). At the level of, IC loss of normal collicular function with age is suggested by a number of studies.(ref) Consideration of these changes is important when examining the auditory system of aged animals since higher acoustical processing is dependent upon the input from these lower auditory structures.

The ability to use the spatial separation between speech and a competing signal has been described in detail (Carhart et. al., '69; Hirsh, '71; Duquesnoy, '83; Gelfand et. al., '88). The ability to perform sentence reception and babble detection thresholds tasks in the presence of competing noise from spatially separated speakers was not impaired in normal aging. On the other hand individuals with presbycusis displayed a significantly smaller binaural advantage than either the young or aged normals (Gelfand et. al., '88). Time-altered speech tests further support the notion that the loss of speech intelligibility seen in elderly subjects may be due to a loss in temporal resolving power of the

central auditory processing system (Konkle et. al., '77). Loss of man's ability to lateralize acoustic signals, a function at least partially attributable to the superior olivary complex (SOC), is associated with age-related loss of speech intelligibility (Herman et. al., '77).

These psychoacoustic findings may reflect age-related changes in the synthesis, degradation, uptake, release, and/or receptor sensitivity of neurotransmitters, perhaps secondary to cell loss. Similar changes could explain certain aspects of the pathology seen in neural presbycusis and with normal aging. The proposed study will examine postulated age-related changes of LSO neurons in response to binaural acoustic stimuli. In a more general sense it has been hypothesized that a variety of age-related differences in cognitive function relate to neural signal-to-noise ratio within the central nervous system. This ratio is reduced with age (Crossman and Szafran, '56; Cremer and Zeef, '87). These authors suggest that signal strength could be degraded by diffuse neuronal loss, by change in the level of inhibition, and/or the noise level could be heightened by increased spontaneous activity.

Table 1. Table showing actual counts and % decrease for both cell number and cell size in mature versus aged fisher-344 rats. Upper values represent 8MA vs. 8AG and lower values represent 13MA vs. 8AG.

Table 2. Table showing results from cresy violet cell counts.

Figure 1. Bar graph showing the number of GABA-labeled neurons in the ventral-lateral portion of the CIC fo 8 mature and

8 aged animals procssed as matched pairs.

Figure 2.A. GABA-immunoreactive neurons are seen scattered throughout the IC in frontal sections of mature (A) and aged (B) Fisher 344 rats.

b. GABA-immunoreactive neurons in the CIC for the same sections as in A.

Figure 3. Schematic drawing (after Paxinos and Watson) showing the center (rostral-caudal) section and the distribution of GABA-immunoreactive neurons from computer generated dot maps in the defined area of the CIC (13 sections from each member of mature/aged pair 7)

A. Distribution of neurons displaying GABA-like immunoreactivity in mature rat 7.

B. Distribution of neurons displaying GABA-like immunoractivity in aged rat 7.

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