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Age-Related Decrease in $GABA_B$ Receptor Binding in the Fischer 344 Rat Inferior Colliculus

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MILBRANDT, J. C., R. L. ALBIN AND D. M. CASPARY. *Age-related decrease in $GABA_B$ receptor binding in the Fischer 344 rat inferior colliculus.* NEUROBIOL AGING **15**(6) 699–703, 1994.—Quantitative receptor autoradiography was used to assess $GABA_B$ receptor binding in three primary subdivisions of the inferior colliculus (IC): dorsal cortex (DCIC), external cortex (ECIC), and the central nucleus (CIC) of 3-, 18–20-, and 26-month-old Fischer 344 rats. $GABA_B$ binding sites were localized using [^{3}H]GABA in the presence of a saturating concentration of isoguvacine, a selective $GABA_A$ receptor agonist, to displace [^{3}H]GABA bound to $GABA_A$ receptor sites. In the three IC subdivisions examined, $GABA_B$ receptor binding was significantly reduced in 26-month-old rats when compared to 3-month-old rats (DCIC, -44%; ECIC, -36%; CIC, -32%; $p < 0.05$). For comparison, $GABA_B$ binding was determined in the portion of cerebellum located in the recess of the IC. In the molecular layer of this region, there were no statistically significant differences in receptor binding between 3, 18–20-, and 26-month-old rats. In addition, there was not a significant age-related change in the cross-sectional area of the IC. These findings provide additional evidence to support the existence of selective age-related changes in GABA neurotransmitter function in the rat IC.

$GABA_B$ GABA Aging Auditory Inferior colliculus Autoradiography Cerebellum

γ-AMINOBUTYRIC acid (GABA) is thought to be the primary inhibitory neurotransmitter in the central nervous system exerting its actions by activation of at least two unique receptor subtypes, $GABA_A$ and $GABA_B$ (5,16). The $GABA_A$ receptor is a multimeric protein related to the super-family of fast, membrane ion channel receptors (28,36,37). $GABA_B$ receptor stimulation can activate K^+-channels, inhibit Ca^{2+}-channels, inhibit or enhance cAMP accumulation, and inhibit phosphotidyl inositol turnover through G protein second messenger systems (4, for review). $GABA_B$ receptors exist both at pre- and postsynaptic sites, as well as on astrocytes (4, for review). Through presynaptic mechanisms, $GABA_B$ receptor activation can modulate the release of a number of different neurotransmitters including both inhibitory and excitatory amino acids (3,4,9,31). Postsynaptic $GABA_B$ receptor activation can result in membrane hyperpolarization, observed as a slow inhibitory post synaptic potential (27).

It has been suggested that altered GABAergic function may contribute to sensory and cognitive disabilities observed in the elderly population (7,24,34,40). Specifically, GABAergic neurotransmission in the inferior colliculus (IC) appears to undergo substantial age-related changes. The IC is a major auditory brainstem structure involved in the processing of acoustic information related to sound localization and the coding of complex signals such as speech (6). The central nucleus of the IC (CIC) in Fischer 344 (F344) rats undergoes a significant age-related decrease in GABA levels, a decreased number of GABA-immunoreactive neurons, a reduction in both basal and K^+-stimulated release of GABA, and a decrease in post-release tissue content of GABA when compared to younger controls (1,7). An age-related reduction in the specific activity of the GABA synthesizing enzyme glutamic acid decarboxylase has been described in the IC of humans and in the CIC of aged F344 rats (24,32).

Previous studies evaluating the effects of aging on GABA receptors have either focused on the $GABA_A$ receptor or did not clearly differentiate between $GABA_A$ and $GABA_B$ sites. Using the selective $GABA_A$ agonist muscimol, investigators have found little evidence of age-related changes in high-affinity receptor binding in the mammalian central nervous system (Table 1). However, nonselective binding studies using radiolabeled GABA suggest an age-related decrease in receptor binding that appears to be regionally specific (Table 1). The goal of this study was to evaluate the effects of aging on $GABA_B$ receptor binding in the F344 rat IC and, for comparison, cerebellum.

METHOD

Tissue Preparation

Seven young adult (3 months), 6 mature (18–20 months), and 7 aged (26 months) F344 rats (Harlan/NIA) were used in this study. Animals were decapitated, brains rapidly removed and fro-

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TABLE 1

AGE-RELATED GABA RECEPTOR BINDING STUDIES

Ligand	Structure	Age-Related Change	Species	References
GABA	Cortex	−23%	SD rat	(21)
		NC	SD rat	(12)
	Cerebellum	−20%	SD rat	(21)
		NC	SD rat	(12)
	Striatum	NC	SD rat	(12)
	Hypothalamus	−39%	SD rat	(12)
	Substantia nigra	−55%	SD rat	(12)
	Midbrain	−24%	SD rat	(21)
	Medulla	−30%	F344 rat	(18)
	Spinal cord	−60%	F344 rat	(18)
Muscimol	Cerebellum	NC	Wistar rat	(23)
		NC	F344 rat/monkey	(39)
		NC	gerbil	(1)
		+35%	mouse	(19)
	Hippocampus	NC	F344 rat	(22)
		NC	F344 rat/monkey	(39)
		NC	guinea pig	(14)
		−24%	gerbil	(1)
	Cortex	NC	guinea pig	(14)
		NC	Wistar rat	(23)
		NC	F344 rat	(25)
		NC	F344 rat	(39)
		NC	SD rat	(10)
		NC	gerbil	(1)
	Thalamus	NC	guinea pig	(14)
		−23%	gerbil	(1)
	Striatum	NC	guinea pig	(14)
		NC	gerbil	(1)
	Substantia nigra	NC	gerbil	(1)
	Inferior colliculus	NC	F344 rat	(26)
	Brain Stem	NC	Wistar rat	(23)

SD = Sprague Dawley; F344 = Fischer 344; NC = No statistically significant change.

zen in Lipshaw embedding matrix on powdered dry ice, and then stored at −80°C until the day of sectioning. Brains were allowed to equilibrate in a cryostat (Minotome, International Equipment Company) and serial transverse sections (20 μm) were cut at −20° to −15°C. Frozen sections were thaw mounted onto chrome-alum/gelatin coated slides and stored at −20°C for no longer than 24 h. Location was verified by counterstaining adjacent sections and comparison of anatomical structures as previously described (11,30).

[^{3}H]GABA Quantitative Autoradiography

$GABA_B$ receptor binding sites were examined using a slightly modified protocol as described (8). Sections were run in triplicate. Sections were prewashed at 4°C in buffer containing 50 mM Tris-HCl and 2.5 mM CaCl2 (pH 7.4 at 4°C) for 30 min and dried under cool air. Sections were then incubated at 4°C for 45 min in 50 mM Tris-HCl and 2.5 mM $CaCl_2$ (pH 7.4 at 4°C) containing 10 nM [^{3}H]GABA (Amersham, 86.5 Ci/mmole) and 10 μM isoguvacine (Research Biochemicals International), a selective $GABA_A$ agonist. This concentration of isoguvacine (10 μM) has been determined to be the ideal concentration to displace [^{3}H]GABA bound to $GABA_A$ receptor sites (8). Nonspecific binding was determined in adjacent sections by the addition of 100 μM (±) baclofen (Research Biochemicals International). Following the incubation, slides were removed individually and rinsed with 3 quick dips in 50 mM Tris-HCl and 2.5 mM $CaCl_2$ (pH 7.4 at 4°C) and 1 quick dip in 2.5% glutaraldehyde in acetone and immediately dried with warm air. Autoradiograms were generated by mounting the slides and a commercial standard containing known amounts of radioactivity (ARC Inc.) in an X-ray cassette, apposed to ^{3}H-sensitive film (Hyperfilm-^{3}H, Amersham) for 3 weeks at 4°C. Films were developed in Kodak D19 for 4 min, fixed for 4 min in Kodak Rapid Fix, washed, and air dried.

Data Analysis

Ligand binding was quantified using computer-assisted densitometry using Bioquant® software. Films were placed on a light box and digitized images were captured using a CCD video camera (Circon). Average optical density was determined by taking multiple density readings from the area of interest, as diagramed in Fig. 2. Density values were converted to fmoles/mg protein using a standard curve generated from the co-exposed ^{14}C-embedded plastic standards. These standards have been previously calibrated against known amounts of tritium and protein (29). Statistical differences in the concentration of bound ligand for each structure and between the age groups were assessed by one-way analysis of variance (ANOVA), and a Tukey-HSD follow-up test, using the statistical program Statgraphics Version 4.0.

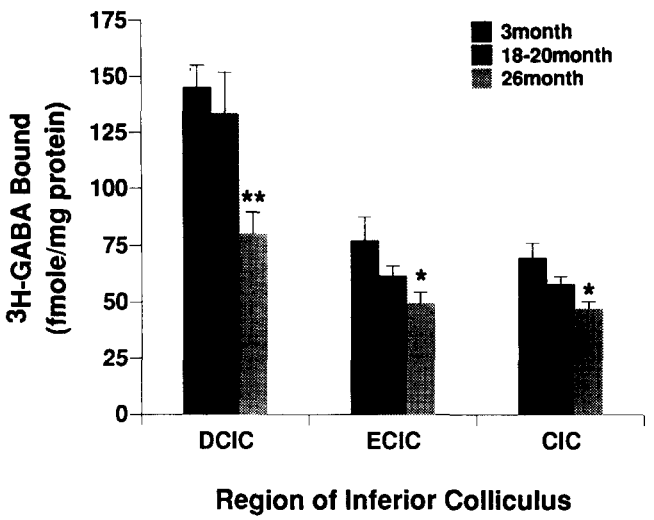

FIG. 1. $GABA_B$ receptor binding in three subdivisions of the inferior colliculus in the F344 rat. Values represent $GABA_B$ receptor binding levels (mean ± SEM) compiled from data obtained in two experiments. In the DCIC, $GABA_B$ receptor binding was significantly reduced in 26-month-old rats when compared to young adults and middle-aged rats (**p = 0.0041). In CIC and ECIC, binding was significantly decreased in 26-month-old rats when compared to young adults (*p = 0.0141 and p = 0.0424, respectively). Binding was significantly higher in the DCIC than the ECIC and CIC ($p < 0.01$) and this difference was consistent across age groups.

RESULTS

$GABA_B$ receptor binding data are shown in Fig. 1 and 2. In the DCIC, $GABA_B$ receptor binding was significantly reduced in 26-month-old rats when compared to young adults (−44%) and 18-20-month-old animals (−40%), $F(2, 17) = 7.732$, $p = 0.0041$. In CIC and ECIC, binding was significantly decreased in 26-month-old rats when compared to young adults (−32% in CIC, $F(2, 17) = 5.53$, $p = 0.0141$ and −36% in ECIC, $F(2, 17) = 3.827$, $p = 0.0424$. $GABA_B$ receptor binding was significantly higher in the DCIC than in the ECIC and the CIC and this difference was consistent across age groups ($p < 0.01$). No other statistically significant differences were observed between the age-groups or the subdivisions of the IC (Fig. 1).

As a comparison, $GABA_B$ binding was also determined in the region of cerebellum located in the recess of the IC. In the molecular layer of this region, no statistically significant difference in receptor binding was observed between young, mature, and aged rats (185.75 ± 23, 202.8 ± 20, and 163.6 ± 17.0 fmoles/mg protein, respectively) (mean ± SEM) (Fig. 2).

To evaluate possible changes in the size of the IC, we determined the cross-sectional area at the level of the CIC, approximately −9.0 Bregma (30). No statistically significant difference was observed between young (7.12 ± 0.28 mm^2), mature (7.04 ± 0.45 mm^2), and aged (7.53 ± 0.26 mm^2) rats.

DISCUSSION

The present study demonstrates a significant decrease in $GABA_B$ receptor binding in the IC of aged versus young F344 rats. This finding is particularly compelling because $GABA_A$ receptor binding appears to be unchanged in the IC in F344 rats (26). In addition, data from the present study and a study in progress (R.L.A.) suggest $GABA_B$ binding is unchanged in the cerebellum of aged rats, thus, deficits may be regionally selective.

The underlying mechanism(s) for the decrease in $GABA_B$ binding is not known, and the present study is unable to determine if the loss is occurring at pre- and/or postsynaptic sites. Preliminary results from an ultrastructural immunocytochemical study suggest a decrease in the number of GABA-immunoreactive and non-immunoreactive presynaptic terminals in the CIC of F344 rats (15). Because significant numbers of $GABA_B$ receptors are thought to be presynaptic and receptor activation has been shown to modulate the release of GABA, glutamate, catecholamines, and neuroactive peptides (3,13,33,35,38), it is possible that the decrease in binding is reflecting a decrease in the number of nerve terminals. Additionally, no statistically significant differences were observed in the cross-sectional area of the IC between age groups. This finding appears to be consistent with previous findings in the IC of aged macaques (17) and mice (41). Currently, saturation analysis studies are in progress to determine if the decrease in $GABA_B$ binding is due to a decrease in number of binding sites and/or an altered affinity for GABA.

It is not apparent whether age-related changes in GABA neurotransmission in the IC are due to changes occurring within the IC

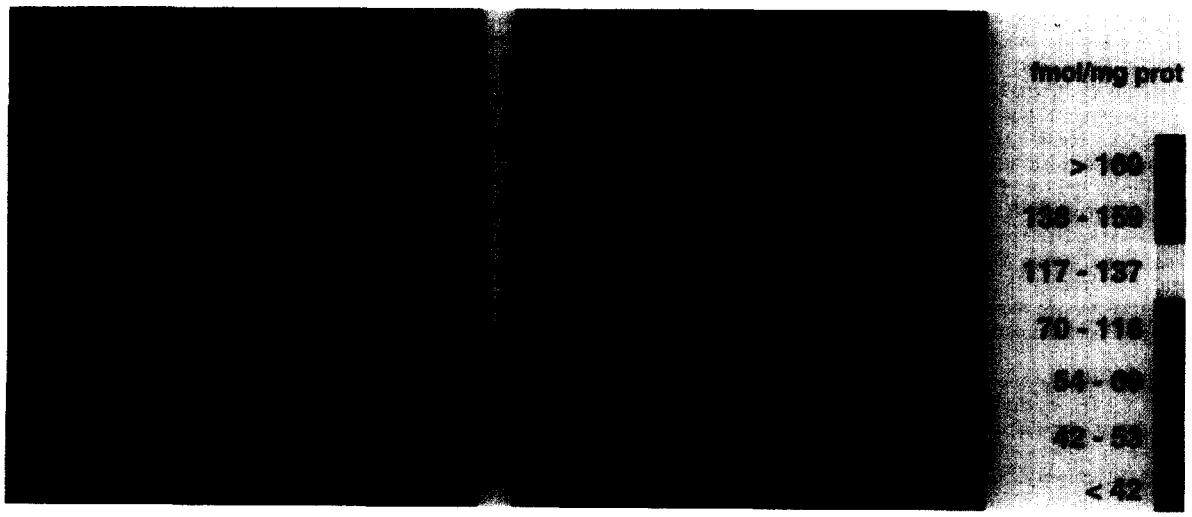

FIG. 2. Autoradiographs of [^{3}H]GABA binding to $GABA_B$-receptor sites in the inferior colliculus (IC) of a representative (A) 3 and (B) 26 month F344 rat. Dotted lines in panel (A) represent the borders of the three subdivisions sampled for data analysis. Color bar on the right indicates color scale for this pair.

or are a direct result of peripheral loss of input. In F344 rats, there is a significant age-related decrease in the density of spiral ganglion cells especially from the basal turn (20). This loss of spiral ganglion cells may result in a decrease in neural input to central auditory structures that could be responsible for the neurotransmitter changes observed in the IC. The present study is unable to determine if the decrease in $GABA_B$ binding is secondary to a peripheral loss of input. Denervation studies in combination with receptor binding may provide answers to these types of questions.

These data, in combination with previous studies showing age-related changes in GABA neurotransmission, suggest the potential for an altered balance between excitation and inhibition in the IC of aged animals. Such an imbalance might alter the ability to process acoustic information. Future physiological and behavioral studies are needed to evaluate in vivo changes associated with the age-related deficits in GABAergic neurotransmission. An understanding of the changes related to GABAergic neurotransmission with age is crucial for the development of compounds which may be useful in the treatment of certain types of hearing and cognitive disorders that affect the elderly population.

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