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ACETYLCHOLINESTERASE AND PSEUDOCHOLINESTERASE IN NEURAL AND NON-NEURAL TISSUE IN THE MEDIAL GENICULATE BODY OF THE CAT*

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Abstract—The technique of retrograde degeneration has shown that acetylcholine esterase is present in both pre- and post-synaptic neurons in the medial geniculate body of cat. In sensory systems the enzyme activity is higher in cell groups than in adjacent fiber tracts. Butyrylcholine esterase is probably present in glial and vascular tissue as well as in neural tissue of the medial geniculate body, and is higher in the fiber tracts of the central nervous system than in most of the adjacent cell groups.

THE distributions of acetylcholine esterase (AChE) and pseudocholine esterase (BChE) in the peripheral and central nervous systems have been described in several species. Using histochemical techniques, Shute and Lewis[1] have studied the cholinesterases in rat brain, and Koelle has studied cholinesterases in both rat brain and cat autonomic ganglia.[2, 3] Giacobini has measured esterases in single cells of the nervous system by Cartesian diver techniques.[4] Burgen and Chipman have measured cholinesterase activities in different areas of dog brain by manometric techniques.[5] Many other studies are referred to in review articles by Koelle and by Giacobini.[6–8] Acetylcholine esterase has been assigned to the pre- and post-synaptic neural tissue, and BChE is believed to be a component mainly of glia and capillary walls. This distribution has been shown most convincingly in the autonomic nervous system and in nerve–muscle preparations. Owing to the complexity of the central nervous system, less is known concerning the cellular location of AChE and BChE. This paper describes the distribution of AChE and BChE in medial geniculate bodies of cat after lesions were made to destroy pre- and post-synaptic neural tissue.[9–11] The distributions of these enzymes in the visual, somatic sensory, and motor systems are also described.

METHODS

Cats were prepared surgically as described elsewhere.[8] Lesions were made of the ipsilateral brachium of the inferior colliculus in conjunction with some cortical lesions. The extent of the lesions was verified when the brains were removed, and all lesions included at least the desired areas. The medial geniculate bodies were dissected

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from the brain stem and from adjacent cell groups, although on histological examination some cells from the magnocellular division were included. The average weight of the experimental medial geniculate bodies was 60·3% of the control weight, with a standard deviation of 13·3%.

Two cats were killed by electroshock six weeks after operation. Their medial geniculate bodies were embedded, sectioned at 10 μ serially, and stained with gold chloride sublimate for astrocytes; silver sodium carbonate for oligodendrocytes and micro- and macroglia; and cresyl violet for both neurons and glia. The control and experimental medial geniculate bodies were compared.

Other animals were anesthetized with pentobarbital sodium (Nembutal), since this drug does not affect choline esterase activity.[12] All animals survived at least six weeks after surgery. The animals were perfused through the aorta with 1 to 2 liters of saline to remove esterases in blood. The brains and retinae were chilled, and the desired regions were dissected out. Samples weighed between 15 and 25 mg. The manometric method of Koelle[13] was used to measure enzyme activities. Each tissue sample was homogenized in 1 ml 0·025 M $NaHCO_3$ and 0·04 M $MgCl_2$, and rinsed with 0·7 ml more. The mixture was placed in the reaction chamber of Warburg vessels; 14 mg acetyl-β-methylcholine bromide or 10 mg butyrylcholine iodide in 0·5 ml of the bicarbonate-magnesium buffer was put into the side arm. Blank tissues were run. The reaction was carried out in 5% CO_2–95% N_2 at 37°. The reactions were run for 0·5 to 1 hr, dependent on the duration of maximal rate. Results are reported in microliters CO_2 per milligram wet weight per hour.

The relative amounts of AChE in the somatic sensory and motor systems and in the control and experimental medial geniculate bodies were not affected by 10^{-8} M di-isopropylfluorophosphate (DFP), although simultaneous control experiments showed that BChE was inhibited 85% to 100% even in areas where this enzyme is most concentrated, and AChE was not affected. It was concluded that under the conditions of this study DFP does not appreciably affect AChE and that BChE does not hydrolyze acetyl-β-methylcholine appreciably.

RESULTS

Figures 1 and 2 show representative sections through the control and experimental medial geniculate bodies stained with cresyl violet. Figure 1 shows the large neurons and relatively sparse glia of control tissue. Figure 2 shows only a few neurons that appear to be normal, and a much greater density of glia. This increase of glial density is probably due to both increase in number and a collapse of the tissue mass. The cell nuclei were counted in photomicrographs, and the ratio of cell density in experimental sections to the density in control sections ranged from 1·8 to 2·4. Figure 3 is a photomicrograph of gold chloride-stained control medial geniculate body, and Fig. 4 an experimental medial geniculate body, both from the same cat. There is a marked proliferation of fibrous astrocytes in the experimental tissue. Silver-stained material showed relatively little change in experimental tissue.

After cortical lesions to remove post-synaptic neurons the AChE activity of the medial geniculate body increased 17% (Fig. 5). When lesions of the brachium of the inferior colliculus were combined with cortical lesions to remove both pre- and post-synaptic neurons, the AChE activity decreased 27% from control. Table 1 shows

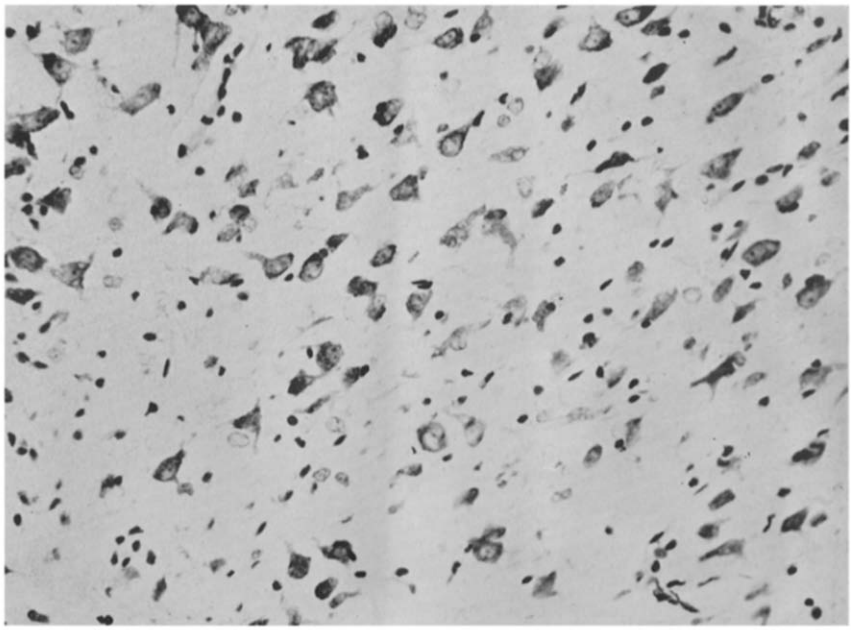

FIG. 1. Section through control medial geniculate body. Cresyl violet stain, section 10 μ thick; 410×.

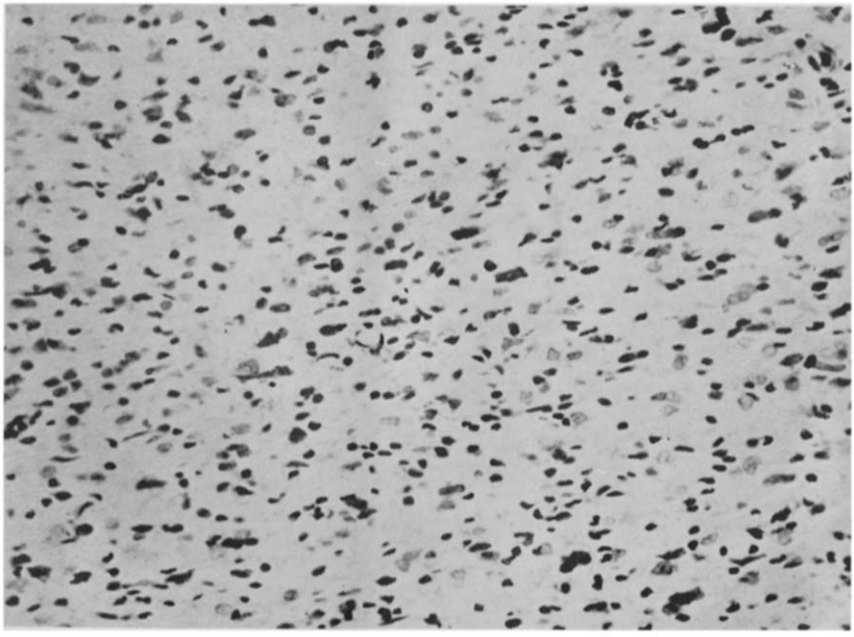

FIG. 2. Section through experimental medial geniculate body. Cresyl violet stain, section 10 μ thick; 410×.

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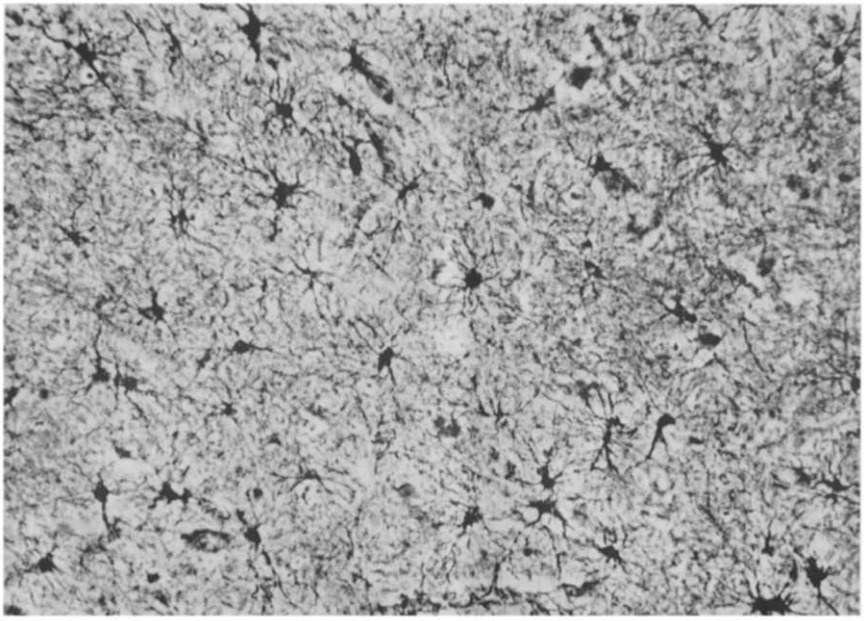

FIG. 3. Section through control medial geniculate body. Gold chloride sublimate stain, section 22 μ thick; 410×.

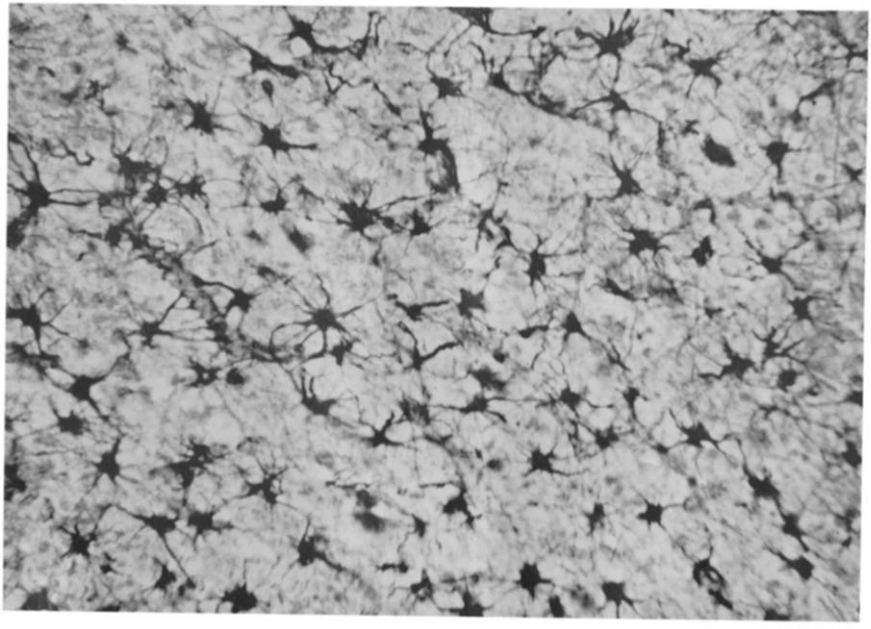

FIG. 4. Section through experimental medial geniculate body. Gold chloride sublimate stain, section 22 μ thick; 410×.

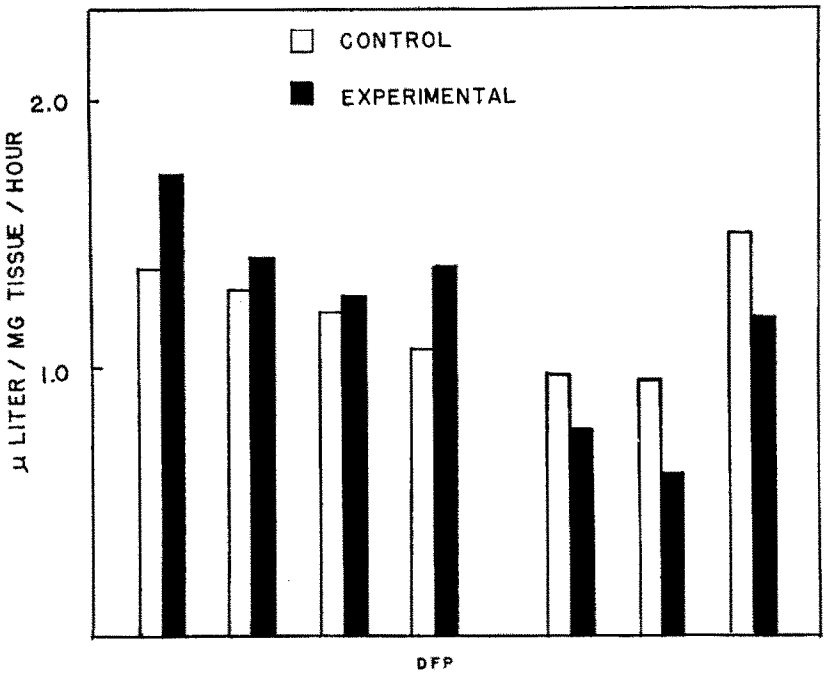

FIG. 5. AChE in the medial geniculate body after cortical lesions alone and in combination with ipsilateral lesions of the brachium of the inferior colliculus. Each pair of bars represents the experimental tissue compared with the contralateral control tissue. The first four pairs of bars are from cats receiving cortical lesions alone; 10^{-8} M DFP was used in the homogenates from one cat, as indicated. The remaining three pairs are from cats receiving both cortical and brachial lesions.

AChE activities in the visual, somatic sensory, and motor systems. The enzyme is lowest in the fiber tracts, relative to adjacent gray areas.

Figure 6 shows that BChE activity is higher than control when pre- and post-synaptic neurons are destroyed. The average increase is 48%, and the combined brachial and cortical lesions do not give a value different from those with the cortical lesion alone. Table 1 shows that BChE activity is higher in the fiber tracts than in the adjacent gray areas, except for the lateral geniculate body.

DISCUSSION

Koch *et al.*[11] removed cortex-receiving fibers from the lateral geniculate body and used that nucleus for their studies of glial ion content. The histology they presented for the normal and degenerated lateral geniculate bodies is similar to that found in the medial geniculate bodies in this study.

The fact that AChE activity increases 17% when post-synaptic neurons are degenerated must mean that there is enzyme in post-synaptic neurons but it is more concentrated in pre-synaptic axon tips, remaining glial and vascular tissue, internuncial cell bodies remaining after the lesion, or a combination of these sites. The combined removal of pre- and post-synaptic neuronal tissue caused a decrease of 27% from control value, or 44% from that value after cortical lesions alone. This large decrease indicates that the greatest concentration of AChE is in the pre-synaptic axon tips.

TABLE 1. AChE AND BChE ACTIVITIES IN THE VISUAL, SOMATIC SENSORY, AND MOTOR SYSTEMS OF THE CAT*

	AChE		BChE			AChE		BChE	
Dorsal columns	3·15 2·23 1·70	(1·70) *2·52*	3·33 3·40 2·05	*2·93*	Retina	2·61 2·12 1·04	*1·92*	0·47 0·55 0·01	*0·34*
Cuneate nuclei	7·20 7·95 5·24	(7·52) *6·79*	6·58 6·41 4·50	*5·83*	Optic tract	0·11 0·57 0·39	*0·35*	2·30 1·84 1·19	*1·74*
Ventral posterior nucleus	1·82 1·23 2·79	(1·60) *1·87*	4·42 3·93 3·80	*4·50*	Superior collic.	6·23 6·03 6·27	*6·17*	6·35 3·39 5·60	*5·11*
Somatic radiat.	1·06 0·22 1·81	(0·63) *0·93*	3·80 3·64 4·19	*3·87*	Lateral genic. body	1·92 2·97 3·74	*2·87*	7·39 3·59 4·43	*5·13*
Somatic cortex	1·68 0·85 1·16	(0·93) *1·16*	1·11 1·36 1·38	*1·28*	Visual radiat.	0·25 0·93 0·41	*0·53*	4·16 2·33 2·79	*3·09*
Motor cortex	1·75 1·40 1·46	(1·13) *1·44*	1·24 1·27 0·84	*1·11*	Visual cortex	0·81 1·01 0·81	*0·87*	1·63 0·54 0·74	*0·97*
Pyramidal tract	1·01 0·00 0·63	(0·05) *0·42*	3·70 3·09 2·38	*3·50*					

* Values are in microliters per milligram tissue per hour. Numbers in parentheses refer to homogenates which contained 10^{-8} M DFP. The italicized figures are the averages for the brain areas.

According to the ideas advanced by Koelle[6, 7] these neurons are probably cholinergic. Shute and Lewis[1] described ventral and dorsal tegmental cholinergic tracts in the rat brain, with the dorsal tract sending fibers to the geniculate bodies as well as to other areas. They reported that AChE diminishes after lesions interrupting fibers in these tracts, and it may be that the brachium of the inferior colliculus of the cat carries such fibers. This would account for the decrease in the AChE in the medial geniculate body after brachial lesions. The fact that the combined lesions did not cause complete loss of enzyme activity indicates that there is AChE either in internuncial or in other neurons unaffected by the lesions, or in glial or vascular tissue. AChE has been reported in glial and vascular tissue in amphibia[12] and in cat.[14] Koenig and Koelle[15] have suggested the possibility that AChE is synthesized in glial cells. Koelle found very little AChE in the pyramidal tracts, neocortex, and optic nerve of the rat, which is consistent with the results reported here for the cat. Both animals have AChE in the retina. While Koelle[7] stated that the AChE increases from the cuneate-gracile nuclei to the lateral thalamic nucleus, a lower activity was found in the ventral posterior nucleus of the cat thalamus. Ord and Thompson[16] reported that human subcortical white matter hydrolyzes less acetyl-β-methylcholine than the overlying cortex, and these results are consistent with those found for the cat. Foldes *et al.*[17] reported similar results in human tissue.

Since lesions of the post-synaptic neurons, alone and combined with lesions of pre-synaptic neurons, cause an increase of BChE in the medial geniculate body, this

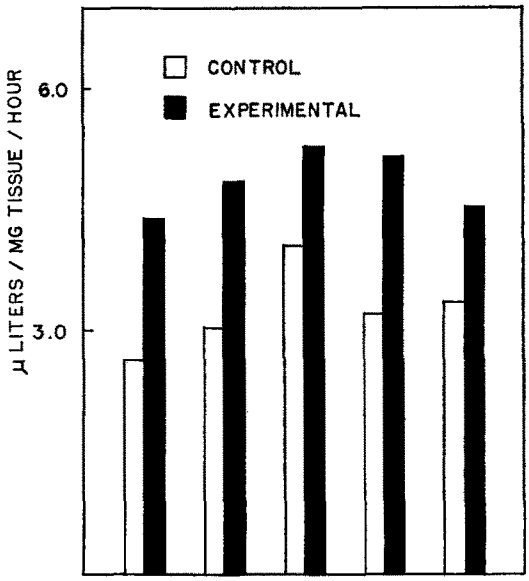

FIG. 6. BChE in the medial geniculate body after cortical lesions alone and in combination with ipsilateral lesions of the brachium of the inferior colliculus. Each pair of bars represents the experimental tissue compared with the contralateral control tissue. The first four pairs of bars are from cats receiving cortical lesions alone. The last pair of bars is from a cat receiving both cortical and brachial lesions.

enzyme must be mainly in the glial cell–capillary wall portion of the tissue, or else in neural tissue not damaged by the lesions. Although Shute and Lewis[1] reported that the dorsal tegmental cholinergic system included fibers containing BChE, the failure of brachial lesions to affect the enzyme level suggests that at least in cat such fibers do not traverse the brachium. Shute and Lewis found BChE in non-neural tissue also. Brightman and Albers[12] concluded that BChE is in the fibrous astrocyte in fiber tracts. Koelle concluded that BChE in the rat central nervous system is confined to capillary walls, vascular smooth muscle fibers, glial cytoplasm (particularly fibrous astrocytes of the tracts), and some motor neurons.[7] While Brightman and Albers and Koelle did not find the enzyme in neural tissue, the increase in specific activity found in the degenerated medial geniculate body of the cat is not as high as it should be if BChE were present only in non-neural tissue. Some of the enzyme must have been removed as a result of the cortical lesions. Furthermore, Abrahams[14] reports BChE in both neural and non-neural tissue of the central nervous system of cat.

Koelle reports that no BChE was observed in the retina of the rat, which is consistent with low activity reported here in the cat. The fiber tracts in the cat have higher BChE activity than the adjacent gray matter except for the lateral geniculate body. The higher BChE activity in these fiber tracts was also reported by Ord and Thompson[16] and Foldes *et al.*[17] in humans.

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