

EARLY SENESECE OF FOREBRAIN CHOLINERGIC SYSTEMS: A GENETIC ANIMAL MODEL

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The morphological organization of putatively cholinergic neurons was studied in the neostriatum of 21-day-old C57BL/6 and DBA/2 mice, by means of acetylcholinesterase (AChE) pharmaco-histochemistry. At this postnatal age no interstrain differences were seen in the organization of AChE-containing neostriatal neurons. The present data indicate that decrease of neostriatal cholinergic neurons, which is present in mature C57BL/6 mice, is not due to a genetically determined different developmental pattern, but rather to cell loss occurring in adult age.

INTRODUCTION

Behavioral studies on inbred mouse strains have demonstrated that C57BL/6 and DBA/2 mice are characterized by opposite open field activity and learning abilities (Bovet et al., 1969; Oliverio, 1983); biochemical studies have also shown significant interstrain differences in the metabolism of acetylcholine (Ebel et al., 1973; Ingram and Corfman, 1980; Pryor et al., 1966), which were suggested to be a correlate of the observed behavioral differences (Mandel et al., 1974). In the attempt to identify the morphological substrate of these biochemical and behavioral variations, we have recently performed a morphological study of cholinergic neurons in adult C57BL/6 and DBA/2 mice (Albanese et al., 1985). We have shown that the number of forebrain cholinergic neurons (located in the medial septum, diagonal band of Broca, basal nucleus of Meynert, and neostriatum) is significantly higher in the DBA/2 strain. In order to evaluate whether these interstrain morphological differences, which have been detected in adult animals, are also present at earlier developmental stages, we have performed a comparative study on 21-day-old mice. We report here that C57BL/6 mice undergo a significant reduction of neostriatal cholinergic neurons from week 3 to 18, which does not occur in the DBA/2 strain:

MATERIALS AND METHOD

Male C57BL/6 and DBA/2 mice were studied at 3 weeks of age by means of a pharmaco-histochemical procedure for acetylcholinesterase (AChE) (Butcher, 1983), which allows a detailed visualization of AChE-containing neurons (Albanese and Butcher, 1980), and is suitable for the study of forebrain putatively cholinergic perikarya (Fibiger, 1982; Satoh et al., 1983). In order to compare histochemically treated specimens, in each experiment the same number of animals from both strains was processed in a parallel fashion. All the animals were injected intramuscularly with an irreversible AChE inhibitor (di-isopropylfluorophosphate, DFP) at the dose of 3.5 mg/kg of body weight. 6 hrs prior to their euthanasia. The mice were sacrificed under deep general

anaesthesia by cardiac perfusion with 0.9 % saline followed by 10% phosphate buffered neutral formalin. The brains were post-fixed in the same formalin solution for 48-72 hrs before being transferred to a cold (4°C) 30% sucrose for an additional 48 hrs period. The brains were cut coronally at 30 μ m intervals according to a standard plane (Sidman et al., 1971). The resulting tissue sections from both strains were processed in parallel according to a standard protocol. They were immersed for 30 mins into 30 μ M N-N'-bis (1-methyl-ethyl)pyrophosphoroamidic anhydride (iso-OMPA), to inhibit butyrylcholinesterase, and then incubated exactly 2 hrs at 21°C in the AChE incubation medium (Butcher et al., 1974). Morphometry was performed by means of computer-assisted image analysis of standard coronal sections of the caudato-putamen (6 rostro-caudal levels). Data from the two strains were compared by analysis of variance.

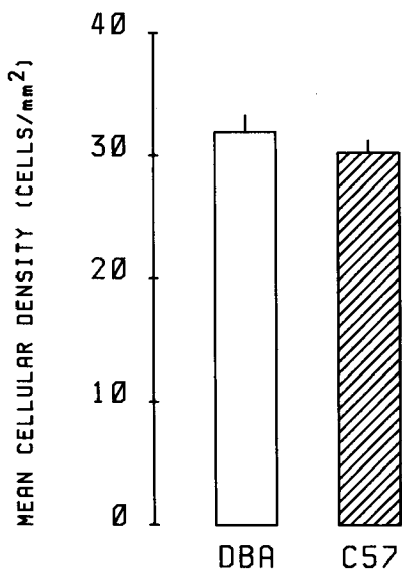


FIGURE 1. Histogram of interstrain differences in the density of AChE-containing perikarya in neostriatum of 21-day-old mice. Data refer to 4 animals (48 sections) of each strain. Boundaries were delineated as they appear in sections counterstained for Nissl's substance. In each animal the right and left hemispheres were analyzed. As no left-right differences were found, values from right and left are shown together.

RESULTS

In both strains, the overall distribution and the morphology of putatively cholinergic neostriatal neurons paralleled that observed in rats by means of histochemical and immunohistochemical techniques (Butcher and Hodge, 1976; Fibiger, 1982; Kimura et al., 1981). In fact, russet AChE-containing neurons, which are believed to represent forebrain cholinergic perikarya (Satoh et al., 1983), were distributed in the caudato-putamen of C57BL/6 and DBA/2 mice. The comparative study did not show significant interstrain differences in the size of neostriatum, nor in the absolute number and the density of colinergic perikarya (Fig. 1). The density of AChE-containing neurons located in the neostriatum of 21-day-old C57BL/6 and DBA/2 mice were, respectively, 30.22 ± 0.96 and

31.89 $\pm$ 1.33, thus very close to values found in 18-week-old DBA/2 mice (Albanese et al., 1985).

DISCUSSION

The present data are in keeping with earlier observations (Butcher and Hodge, 1976) indicating that the appearance of AChE-containing neurons in 21-day-old rats is similar to that observed in mature animals. When comparing the present data to our previous observations (Albanese et al., 1985), it is observed that in C57BL/6 mice a significant reduction of neostriatal cholinergic neurons occurs from postnatal weeks 3 to 18. In fact, in this strain neural density decreases by approximately 20% ($P < 0.001$). Contrastingly, neural density in neostriatum of DBA/2 mice does not show significant variations from week 3 to 18 (Fig. 2). Therefore, the present findings indicate that, while development of cholinergic neurons in C57BL/6 and DBA/2 mice is similar, a significant cell loss occurs in the C57BL/6 strain during adult age. This process is dependent upon the genetic make-up and its time course is presently unknown; to this respect, developmental studies are currently in progress in our laboratories.

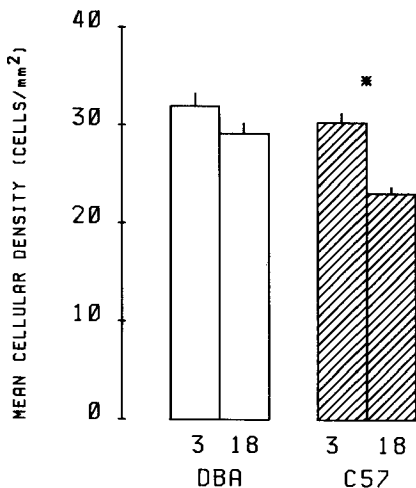


FIGURE 2. Comparative histogram of the densities of AChE-containing perikarya in the neostriatum of 3- and 18-week-old mice. It is shown that the number of AChE-containing neostriatal neurons does not significantly decrease in the DBA/2 strain, as it does in the C57BL/6 strain. Abbreviations: 3, 18 indicate weeks of age; * = $P < 0.001$.

The present data may have some important clinical implications. It has been recently suggested that Alzheimer's disease, a degenerative pathology of human brain which primarily affects memory and cognitive functions, is correlated to a reduction in choline acetyltransferase and AChE in the forebrain (Terry and Davis, 1980). Morphological studies have further demonstrated a significant depletion of cholinergic perikarya occurring in mature age (Nagai et al., 1983;

Whitehouse et al., 1982).

The etiology of Alzheimer's disease is still unknown, however it is relevant to remind that the existence of genetic markers specific to this degenerative disease has been also demonstrated (Nerl et al., 1984). Therefore, it is possible that the study of C57BL/6 and DBA/2 mice might constitute a suitable animal model for the understanding of human pathology affecting forebrain cholinergic transmission.

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