NEUROANATOMY OF AGING BRAIN: SENSITIVITY TO PHARMACOLOGICAL TREATMENT

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SUMMARY

The influence of aging and of nucleus basalis magnocellularis (NBM) lesions on the morphology of the rat hippocampus were assessed using neurohistological techniques and the histochemical method for the detection of zinc tissue stores (sulfide-silver staining technique). Moreover, the effect of choline alfoscerate treatment on the changes induced by aging and by NBM lesioning was investigated.

Aging was characterized by a loss of hippocampal neurons which occurs primarily between middle (12 months) and old (24 months) age, as well as by a reduction of the area occupied by mossy fibres and their density as revealed by sulfide-silver staining. NBM lesions induced primarily a decrease in the density of hippocampal mossy fibres as early as 3 weeks after lesioning.

In choline alfoscerate-treated old rats the number of hippocampal neurons, the area occupied by mossy fibres and their density of sulfide-silver staining are significantly higher than in age-matched controls. The same is true for the density of this staining in the mossy fibres of NBM-lesioned rats.

These findings suggest that morphological impairment of the rat hippocampus caused by aging or NBM lesioning could be sensitive to pharmacological treatment.

INTRODUCTION

The hippocampus plays an important role in regulating learning and memory processes. Moreover, it is involved in the mechanisms responsible for the elaboration of selective attention by eliminating irrelevant information[1-3].

The occurrence of age-related changes in hippocampal structure and function is well documented[4]. These modifications have been put in relationship with the pathogenesis of learning and memory disturbances rather common in old age[5]. Moreover, significant correlations have been demonstrated between cognitive behaviour impairment and structural changes of the hippocampus produced experimentally or induced by aging[4,6]. We have recently demonstrated that lesions of the nucleus basalis magnocellularis (NBM), which is a basal telecephalic cholinergic nucleus sending projections to the fronto-parietal cortex, induce neurodegenerative effects on the intrahippocampal associative pathway of mossy fibres[8].

The present study was designed to evaluate whether some rather specific structural changes of aging rat hippocampus such as nerve cell loss and impairment of mossy fibres were sensitive to pharmacological treatment. Moreover, we looked at whether the degenerative changes of mossy fibres induced by NBM lesions were sensitive to pharmacological treatment.

MATERIALS, METHODS AND STATISTICS

Aging

Male Sprague Dawley rats of 3 months (n =10, considered to be young), of 12 months (n =16, considered to be adult) and of 21 months (n =22, considered to be senescent) were obtained by Charles River Italy. Sensecent animals were randomly allotted to two groups of 16 rats each. The rats of the first group did not receive any treatment and were used

as controls. Animals of the second group received a daily oral dose of 100 mg/Kg/day for 3 months. Senescent rats were killed at 24 months. At this age they were considered to be old.

Ten rats per group (young, adult, old and old plus treated) were anaesthetized with ether and were perfused with a 4% formalin solution to fix the brain. At the end of perfusion, brains were removed, fixed for an additional week and embedded in Historesin according to the procedure described in an earlier paper[9]. Serial sections including the hippocampal formation were cut using a motorized microtome, mounted on microscrope slides and stained with toluidine blue. Five consecutive sections 25 mm apart of each brain were used to count the number of pyramidal neurons of the CA-1 and CA-3 field of the hippocampus and of granule neurons of the dentate gyrus. Counts were made on a 0.5 mm^2 area of the hippocampal regions examined at a final magnification of x 100 according to the procedure detailed elsewhere[9].

Six adult, aged and aged plus treated rats were perfused with a sodium sulfide solution and then processed to demonstrate stores in zinc and other heavy metals in the mossy fibres using the sulfide-silver staining technique. Details on the procedure used for sulfide-silver staining technique as well as for measuring the area occupied by mossy fibres and the density of the staining within the CA-3 and CA-4 fields of mossy fibres are reported in our previous papers[10,11].

Nucleus basalis magnocellularis lesions

Eighteen male Sprague Dawley rats (Charles River Italy) of 4 months of age were divided into 3 groups of 6 animals each. Animals were put under pentobarbital anaesthesia in a stereotaxic frame and injected into the right NBM (for coordinates see[8]) with 1 ml of a 0.9% NaCl solution containing (lesioned rats, n =12) or not containing (sham operated rats, n =6) 10 mg ibotenic acid. Lesioned animals were divided into two groups of 6 rats each. Animals of the first group did not receive any treatment and were used as controls. The remaining rats received a daily oral dose of 100 mg/kg choline alfoscerate starting from the 24th hour after NBM lesioning.
Four weeks after surgery the right hemisphere of lesioned animals was treated with the sulfide-silver staining technique mentioned above.

Quantitative assessment of the area occupied by mossy fibres and of the density of the staining was done as mentioned in the aging section.

Statistics

Values for individual animals within the age groups were means of parameters examined. Group means were calculated from these means. The values of the number of neurons in the 3 hippocampal areas investigated, of the area occupied by mossy fibres and of the density of sulfide staining were evaluated as repeated values of dependent variables. Data were then compared by analysis of variance (ANOVA) or of covariance (ANOCOVA, for the density of sulfide silver staining only using the value of the optical density of corpus callosum as covariate), taking as level of significance a probability of < 0.05.

RESULTS

Aging

The number of nerve cells in the CA-1 and CA-3 fields of hippocampus and in the dentate gyrus is shown in Figure 1. As can be seen, the number of pyramidal neurons of the CA-1 field and of granule neurons of the dentate gyrus is unchanged between young and adult rats, but it is significantly reduced in old rats (Figures 1 and 2). Pyramidal neurons of the CA-3 field show a gradual age-dependent loss (Figures 1 and 3). In choline alfoscerate-treated rats the number of pyramidal neurons of the CA-1 and CA-3 fields and of granule neurons of the dentate gyrus was significantly higher than in age-matched control rats (Figures 1-3). In agreement with the results of previous studies from our group[10,11] a decrease both in the area occupied by mossy fibres (data not shown) and in the density of sulfide-silver staining at the level of mossy fibres was noticeable between adult and old rats (Figure 4). Choline alfoscerate treatment induced an increase of the values of the area occupied by mossy fibres and of the intensity of sulfide silver staining in aged rats (Figure 4).

Fig. 1. Effect of aging and of choline alfoscerate treatment on the number of pyramidal neurons in the CA-1 (CA1) and CA-3 (CA3) fields and of granule neurons in the dentate gyrus (GD) of the rat hippocampus. Values represent the number of nerve cells ± S.E. per 0.5 mm^2 of tissue area. Neurons were counted as indicated in the materials and methods section. Young = young rats; Adult = adult rats; Old = old rats; Old + GFC = choline alfoscerate-treated old. a=P<0.05 vs. young; b=P<0.001 vs. young or adult; c=P<0.001 vs. young and P<0.001 vs. adult; e=P<0.01 vs. old; f=P<0.05 vs. old; g=P<0.02 vs. old.

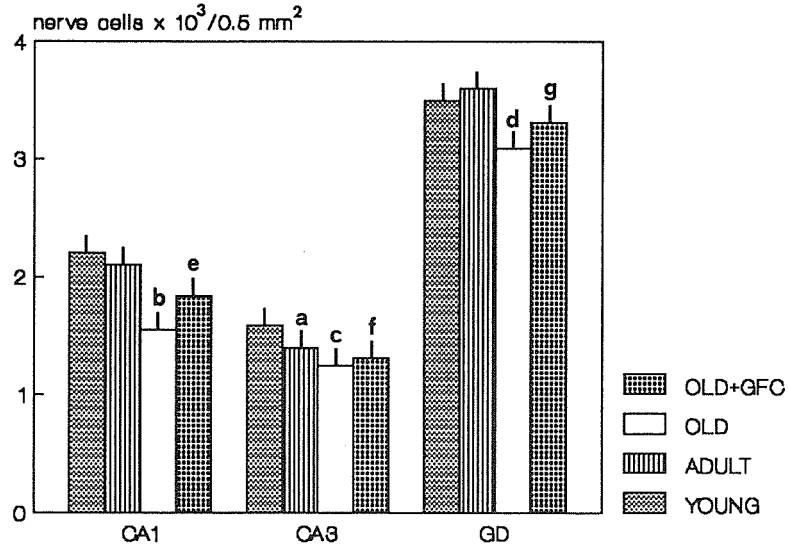


Fig. 2. Rat hippocampus. CA-1 field. Toluidine blue. A = Adult rat; B = Old rat; C = Choline alfoscerate-treated old rat. Note the reduced density of nerve cells in old in comparison with adult rat. The density of nerve cells is higher in choline alfoscerate-treated than in age-matched control animals. (x 120).

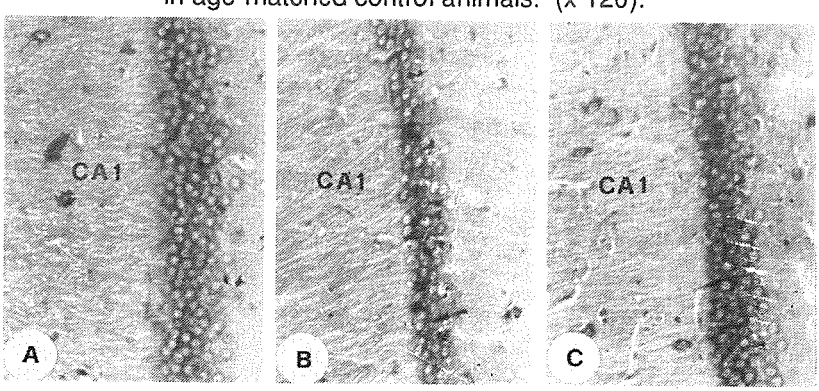


Fig. 3. Rat hippocampus, CA-3 field. Toluidine blue. A = Adult rat; B = Old rat; C = Choline alfoscerate-treated old rat. (x 140).

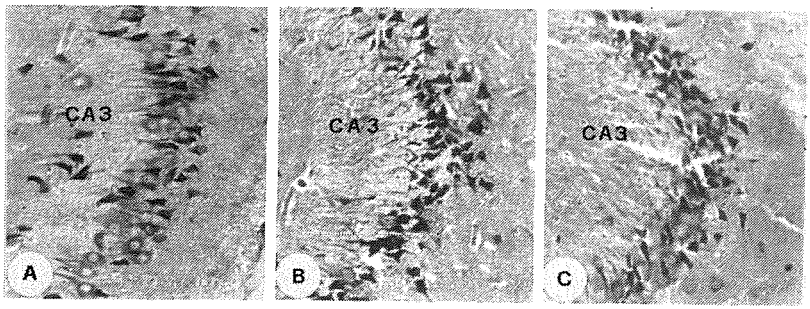
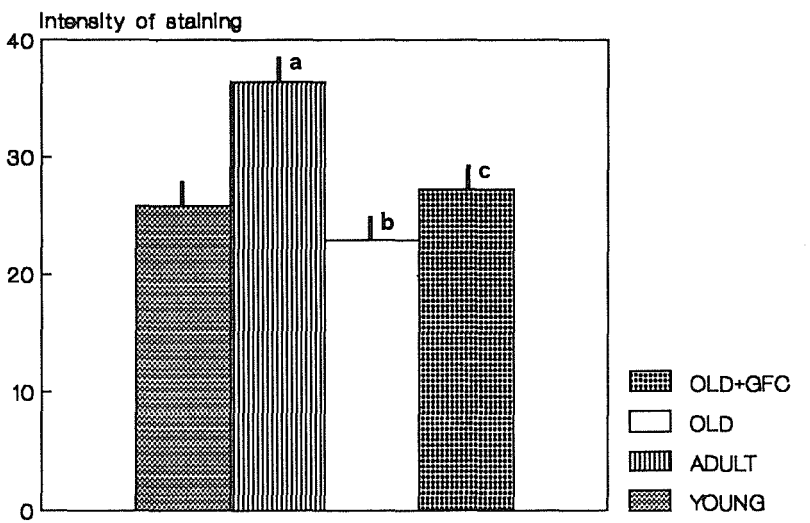


Fig. 4. Density of sulfide silver staining in the hippocampal mossy fibres (CA-3 field) in young, adult, old and choline alfoscerate-treated old (Old+GFC) rats. The values are expressed in arbitrary units ± S.E. and were obtained as described in the materials and methods section. a = P<0.001 vs. young; b = P<0.001 vs. adult and P<0.01 vs. young; C = P<0.01 vs. old and P<0.001 vs. adult.

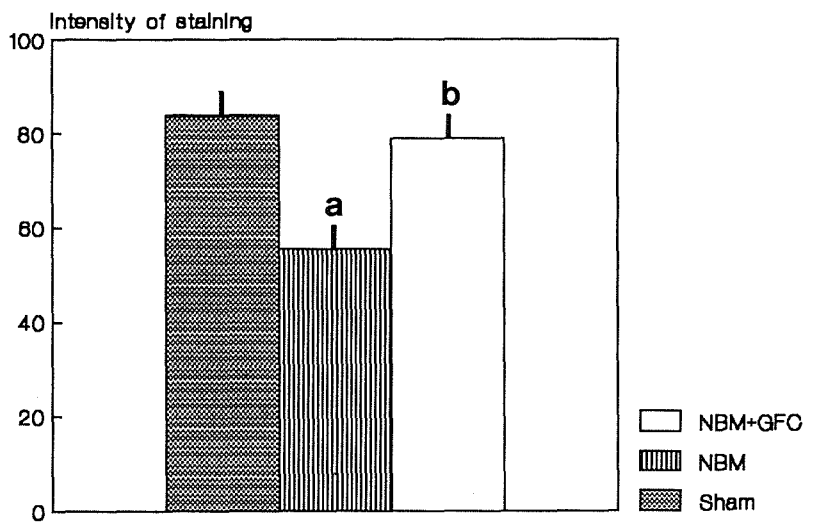


Nucleus basalis magnocellularis lesions

Four weeks after NBM lesioning, the values of the area occupied by mossy fibres were not significantly different between sham-operated

and NBM-lesioned rats (data now shown). In contrast, the intensity of sulfide-silver staining in the CA-3 and CA-4 fields of mossy fibres was significantly reduced (Figures 5 and 6) in NBM-lesioned in comparison with sham-operated rats. Choline alfoscerate treatment was without effect on the area occupied by mossy fibres, but partly restored the intensity of sulfide-silver staining mossy fibres (Figures 5 and 6).

Fig. 5. Density of sulfide silver staining in the hippocampal mossy fibres (CA-3 field) in sham operated (Sham), Nucleus Basalis Magnocellularis lesioned (NBM) 4 weeks after lesioning and choline alfoscerate-treated Nucleus Basalis Magnocellularis lesioned (NBM + GFC) rats. The values are expressed in arbitrary units ± S.E. and were obtained as described in the materials and methods section. a = P<0.001 vs. sham; b = P<0.001 vs. lesioned.



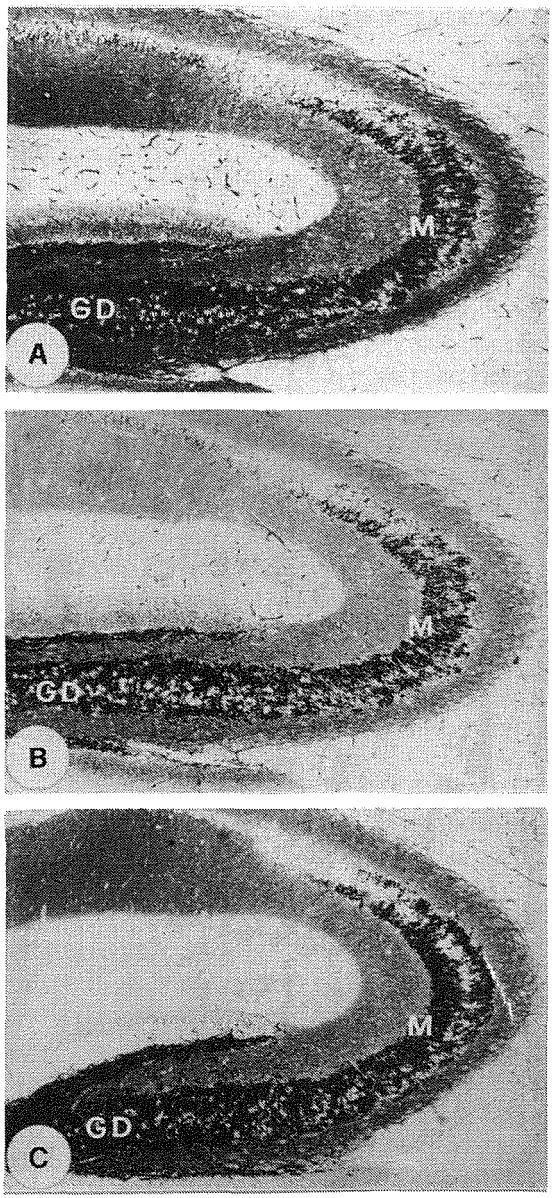


Fig. 6. Sections of rat hippocampus processed with the sulfide silver technique.
A = Sham-operated rat;

B = Nucleus Basalis Magnocellularis lesioned rat, 4 weeks after lesioning;

C = Choline alfoscerate-treated Nucleus Basalis Magnocellularis lesioned rat.

GD = dentate gyrus;
M = mossy fibres.

Note the reduced density of the staining in the mossy fibres area in the lesioned rat and the recovery induced by choline alfoscerate treatment.
(x 85).

DISCUSSION AND CONCLUSIONS

The purpose of the present study was to analyze whether pharmacological treatment with choline alfoscerate was able to counter some anatomical changes occurring in the rat hippocampus with aging and after experimental cerebral lesions. Choline alfoscerate is a compound which increases the bioavailability of choline in the nervous tissue[12] and is a precursor in the synthesis of several brain phospholipids[13-15].

To assess the sensitivity of choline alfoscerate treatment on the anatomy of aging hippocampus, two parameters were analysed, the density of nerve cells in some hippocampal fields and the morphology of the intrahippocampal pathway of mossy fibres. The occurrence of an age-dependent loss of nerve cells is an important and rather extensively investigated parameter of aging brain. There is a good agreement between investigators of a neuronal loss in the mammalian species including man[4,6,9]. The topic of the occurrence of age-dependent reductions of granule neurons of the dentate gyrus is controversial[4,16-18], although the most recent investigations reported a loss of this type of nerve cells in aged male Sprague-Dawley rats[4]. In addition to confirming the loss of the three different nerve cell populations investigated in aged rats, the present study showed that long term choline alfoscerate is able to counter, this phenomenon in part.

As mentioned above, mossy fibres represent an intrahippocampal associative pathway. They are constituted by the axons of granule cells of the dentate gyrus and end mostly on the pyramidal neurons of the CA-3 field[19,20]. Mossy fibres have a high concentration of zinc and other transitional heavy metals and can be easily visualized with techniques for the demonstration of zinc and transitional metals such as the so called sulfide-silver staining technique[21]. From a functional point of view mossy fibres are probably involved in the elaboration of passive avoidance responses[22].

By using specific techniques for the histochemical detection of mossy fibres we have demonstrated that they are impaired between middle and old age[10] and that changes appear starting from the 3rd week after NBM lesioning[8]. The present study confirms:

(a) the occurrence of impairment of the mossy fibres system in the hippocampus of aged and NBM-lesioned rats;

(b) that choline alfoscerate treatment was able to consider these changes. The density of sulfide silver staining is probably in relationship with the density of zinc or of heavy metals containing synaptic buttons[19,20]. It cannot be excluded that the effects of choline alfoscerate on hippocampal mossy fibres may represent the expression of modified synaptic density in this associative pathway elicited by the compound.

In conclusion, these findings suggest that appropriate neuroanatomical and neurohistochemical techniques could be used not only for investigating brain structure in aging or in models of degenerative cerebral disorders, but also for assessing the effectiveness of pharmacological compounds on aging brain.

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REFERENCES

1. Seifert W (1983). Neurobiology of the Hippocampus. Academic Press, London.
2. Isaacson RL and Pribram KH (1985). The Hippocampus. Plenum Press, New York, Vol. 1 and 2.
3. Douglas RM and Goddard G (1975). Long-term potentiation of the perforant path-granule cell synapse in the rat hippocampus. Brain Res., 86: 205-215.
4. Landfield PW, Pitler TA and Applegate MD (1986). The aged hippocampus. A model system for studies on mechanisms of behavioural plasticity and brain aging. In: The Hippocampus. (Editors: RL Isaacson and KH Pribram). Vol. 3 pp. 323-367. Plenum Press, New York.
5. Bartus RT, Dean RL, Goas JA and Lippa AS (1980). Age-related changes in passive avoidance retention: modulation with dietary choline. Science, 209: 301-303.
6. Kadar T, Silverman M, Brandeis R and Levy A (1990). Age-related structural changes in the rat hippocampus: correlation with working memory deficiency. Brain Res., 512: 113-120.
7. Smith G (1988). Animal models of Alzheimer's disease: experimental

cholinergic denervation. Brain Res. Rev., 13: 103-118.
8. Amenta F, Bronzetti E, Caporali MG, Ciriaco E, Germana GP, Niglio T, Ricci A and Scotti de Carolis A (1991). Nucleus basalis magnocellularis lesions impair mossy fiber system in rat hippocampus: a quantitative histochemical and ultrastructural study. Arch. Gerontol. Geriatr., 12: 49-58.
9. Napoleone P, Ferrante F, Ghirardi O, Ramacci MT and Amenta F (1990). Age-dependent nerve cell loss in the brain of Sprague-Dawley rats: effect of long term acetyl-L-carnitine treatment. Arch. Gerontol. Geriatr., 10: 173-185.
10. Ricci A, Ramacci MT, Ghirardi O and Amenta F (1989). Age-related changes of the mossy fibre system in the rat hippocampus: Effect of long term acetyl-L-carnitine treatment. Arch. Gerontol. Geriatr., 8: 63-71.
11. Amenta F, Jaton AL and Ricci A (1990). Effect of long term hydergine treatment on the age-dependent loss of mossy fibers and of granule cells in the rat hippocampus. Arch. Gerontol. Geriatr., 10: 287-296.
12. Mann SP and Bennet RC (1979). The fate of choline in the circulating plasma of the rat. Experientia, 35: 211-212.
13. Smith SW, Weiss SB and Kennedy EP (1957). The enzymatic dephosphorylation of phosphatidic acids. J. Biol. Chem., 228: 915-921.
14. Stein Y and Shapiro B (1957). The synthesis of neutral glycerides by fractions of rat liver homogenates. Biochim. Biophys. Acta, 24: 197-209.
15. Abbiati G, Arrigoni M, Longoni A, Lachman G and Bergamaschi M (1990). Farmacocinetica e metabolismo di 14-C colina alfoscerato nel ratto. Basi Raz. Ter., 20, S.1: 1-11.
16. Geinisman Y, Bondareff W and Dodge JT (1977). Partial differentiation of neurons in the dentate gyrus of the senescent rat. Brain Res., 134: 541-545.
17. Geinisman Y, Bondareff W and Dodge JT (1978). Dendritic athrophy in the dentate gyrus of the senescent rat. Amer. J. Anat., 152: 321-330.
18. Coleman PD, Flood DG and West MJ (1987). Volumes of the components of the hippocampus in the aging F344 rat. J. Comp. Neurol., 266: 300-306.
19. Amaral DG, Dent JA (1981). Development of the mossy fibers and their expansions. J. Comp. Neurol., 195: 51-86.
20. Claiborne BJ, Amaral DG, Cowan WMA (1986). A light and electron microscopic analaysis of the mossy fibers of the rat dentate gyrus. J. Comp. Neurol., 246: 435-458.
21. Haug FMA (1967). Electron microscopical localization of the zinc in hippocampal mossy fibre synapse by a modified sulfide silver procedure. Histochemie, 8: 355-368.
22. Schwegler H, Lipp HP, Van der Loss H and Buremalter H (1981b). Individual hippocampal mossy fibre distribution in mice correlates with two-way avoidance performance. Science, 214: 817-819.
23. Martinez-Guijarro FJ, Molowny A and Lopez-Garcia C (1987). Timm-staining intensity is correlated with the density of Timm-positive presynaptic structures in the cerebral cortex of lizard. Histochemistry, 86: 315-319.