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Lipid Peroxides in Brain During Aging and Vitamin E Deficiency: Possible Relations to Changes in Neurotransmitter Indices

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NODA, Y., P. L. McGEER AND E. G. McGEER. *Lipid peroxides in brain during aging and vitamin E deficiency: Possible relations to changes in neurotransmitter indices.* NEUROBIOL. AGING 3(3) 173–178, 1982.—Lipid peroxide levels, were found to be significantly higher in brains of 18 month old as compared to 4 month old rats, with particularly large increases occurring in the olfactory bulb, globus pallidus, cerebral cortex and caudate-putamen (CP). Eighteen month old rats fed a vitamin E deficient diet for 9 months before sacrifice had lipid peroxide levels significantly higher than age-matched controls in the cerebral cortex, hippocampus and hypothalamus. Age-related decreases were seen in choline acetyltransferase, acetylcholinesterase and ^{3}H-QNB binding in some but not all brain regions, while GABA transaminase and MAO showed age-related increases. No age-related change was seen in tyrosine hydroxylase in the CP or in ^{3}H-dihydroalprenolol (DHA) or ^{3}H-spiroperidol binding in the cortex. As compared with controls, vitamin E deficient rats showed decreases of 38% in cortical ^{3}H-DHA binding, of 33% in ^{3}H-QNB binding in the CP and of 23% and 12% in choline acetyltransferase in the CP and cerebellum, respectively. There were no completely consistent regional correlations between significant changes in lipid peroxidase levels and any neurotransmitter indices studied except for MAO which was only measured in the caudate-putamen.

Lipid peroxidase Vitamin E deficiency Aging Cholinergic systems Cerebral cortex
Hippocampus Hypothalamus Olfactory bulb Globus pallidus Caudate-putamen GABA-T MAO

FREE radicals are constantly being formed in various reactions in biological systems and are considered as possibly important factors in aging. Harman hypothesized that aging and the associated degenerative diseases are attributable to the deleterious reactions of free radicals with cell constituents and connective tissues, and postulated a beneficial role for antioxidants as inhibiters of such processes [12,13]. The accumulation of lipofuscin, which is considered a polymerization product of autooxidized polyunsaturated fatty acids, is one of the most consistent morphological changes in the nerve cells of aged organisms [6, 26, 36]. Its significance, however, to the aging process is not yet clearly understood. Vitamin E is recognized as a biological antioxidant which may protect polyunsaturated lipids against peroxidative chain reactions [5, 9, 21, 43–46]. After chronic vitamin E deficiency, central and peripheral nervous system lesions have been observed in rats and monkeys [30,33], and both an increased accumulation of lipofuscin [16, 17, 29, 42] and some deterioration of learning and memory [17] have been reported in rats. On the other hand, vitamin E failed to prevent the decline of learning ability with aging in mice, although brain lipofuscin content was decreased by the vitamin E supplementation [11].

Numerous neurochemical studies have revealed age-associated changes in neurotransmitter enzyme activities and receptor binding in experimental animals as well as humans [23,35]. The dysfunctions of brain with advanced age may be partly due to these alterations in neurotransmitter systems, which occur, in particular, in certain brain regions in some neurological disorders of advancing age: these include senile dementia, Parkinson's disease and Huntington's disease. So the question arises as to whether deficiency of the antioxidant, vitamin E, might accelerate these age-related neurochemical alterations. In the present study, both lipid peroxidation and some indices of certain neurotransmitter systems have been examined after long-term vitamin E deficiency and in young vs. old rats.

METHOD

Male Wistar rats were used for the experiments. Nine

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month old animals were divided randomly into two groups. The first group was fed normal Purina Rat Chow, known to contain 66 mg of α-tocopherol per kg of diet. The second group was fed a tocopherol deficient diet purchased from ICN Pharmaceuticals, Inc. These treatments were continued for nine months until sacrifice. Four month old rats of the same strain, which had been fed with normal Purina Rat Chow, were sacrificed at the same time. Brains were frontally sectioned into 500 μm thick slices under nitrogen in ice-cold saline and the olfactory bulb, globus pallidus, caudate-putamen, cerebral cortex, hippocampus, thalamus, substantia nigra, cerebellum and spinal cord (C1-C2) were dissected from the slices by the previously described technique [31]. All samples were stored at −80°C until assayed.

Each tissue sample was sonicated in a Polytron homogenizer (setting 5 for 30 sec) in ice-cold distilled water saturated with nitrogen.

Lipid peroxidation was quantitated by fluorometric determination of malondialdehyde (MDA), the end product of lipid peroxidation [32]. A portion of homogenate equivalent to 500–900 μg protein was solubilized with 0.2 ml of 8.1% sodium dodecylsulfate (SDS) solution. Subsequently 1.5 ml of 20% acetic acid-NaOH buffer, pH 3.5, and 2.1 ml of an 0.57% solution of thiobarbituric acid were added. The reaction mixture was heated in boiling water for 60 min. After cooling, 4 ml of n-butanol-pyridine (15:1 v/v) was added and the mixture shaken vigorously. After centrifugation at 1,500 × g for 15 minutes, fluorescence in the organic layer was measured at excitation 515 nm and emission 553 nm on an Aminco Bowman fluorescent spectrophotometer. 1,1,3,3-Tetraethoxypropane was used as the standard for MDA and the lipid peroxide level was expressed as nmoles MDA per g protein. Protein was measured according to the method of Lowry *et al.* [19].

All of the neurotransmitter enzyme activities were assayed radioenzymatically on portions of the aqueous homogenates. Briefly, choline acetyltransferase (ChAT) activity was determined according to the method of Fonnum [10], with 20 μl of tissue homogenate being incubated for 15 minutes at 37°C in a medium containing 50 mM sodium phosphate buffer, pH 7.4, 0.1 mM physostigmine, 80 mM choline chloride, 300 mM NaCl, 20 mM EDTA and 20 μM [1-^{14}C]acetyl coenzyme A (Amersham, specific activity 56.6 mCi/mmol). The reaction was terminated by adding 5 ml of ice-cold 10 mM sodium phosphate buffer, pH 7.4, followed by 2 ml of acetonitrile containing 10 mg tetraphenylboron, and 10 ml of a toluene scintillation cocktail. The ^{14}C-acetylcholine formed was extracted into the organic phase and counted.

Acetylcholinesterase (AChE) activity was assayed according to the method of Sterri and Fonnum [38]. The original tissue homogenate was diluted with 25 mM sodium phosphate buffer pH 7.4 containing 0.5% Triton X-100. The incubation was carried out in a scintillation vial for 15 minutes at 30°C with 20 mM sodium phosphate buffer pH 7.4, 0.25% Triton X-100, 0.1 mM ethopropazine, and 1.3 mM [1-^{14}C]acetylcholine (NEN specific activity 5 mCi/mmol) in a total volume of 70 μl. Radioactive acetate formed in the reaction was extracted for counting into 10 ml of scintillation mixture in the presence of 4 ml of 0.2 M tri-n-octylammonium phosphate in isoamyl alcohol.

Glutamic acid decarboxylase (GAD) activity was determined by a modification of the method of Alberts and Brady [1]. The incubation was carried out for 30 minutes at 37°C in 20 mM potassium phosphate buffer, pH 7.4, 0.1 mM pyridoxal phosphate, 0.02% bovine serum albumin, 2.0 mM L-[1-^{14}C]glutamate (NEN 50.0 mCi/mmol). The reaction was terminated by the injection of 0.2 ml of 2 M H_2SO_4. The $^{14}CO_2$ formed was trapped by hyamine hydroxide.

GABA transaminase (GABA-T) activity was measured according to the method of Sterri and Fonnum [38]. The tissue homogenate was incubated for 30 minutes at 30°C in a medium containing 50 mM Tris-HCl buffer pH 8.6, 0.2% Triton X-100, 1 mM succinate, 0.3 mM pyridoxal phosphate, 3 mM NAD, 10 mM dithiothreitol, 2.5 mM 2-ketoglutarate and 5 mM 4-amino-[U-^{14}C]-butyric acid (NEN specific activity 213.0 mCi/mmol) in a total volume of 70 μl. The radioactive succinate formed was extracted directly by the same procedure as in the AChE assay.

Tyrosine hydroxylase (TH) activity was determined according to the method of Waymire *et al.* [48]. Tissue homogenate was incubated for 20 minutes at 37°C in a medium containing, in a total volume of 250 μl, 200 mM sodium acetate buffer pH 6.1, 1 mM ferrous sulfate, 2 mM dimethyl-tetrahydrobiopterin ($DMPH_4$), 40 mM 2-mercaptoethanol, 2 mM sodium phosphate, 10 μM pyridoxal phosphate, 7.5 units of aromatic L-amino acid decarboxylase obtained from hog kidney and 0.1 mM l-^{14}C-tyrosine (NEN specific activity 56.6 mCi/mmol). The $^{14}CO_2$ formed by the coupled decarboxylation of ^{14}C-dopa synthesized from L-[1-^{14}C]tyrosine was trapped in the hyamine hydroxide and measured.

Monoamine oxidase (MAO) activity was determined according to the method of Wurtman *et al.* [50]. Tissue homogenate was incubated for 20 minutes at 37°C in 0.5 M phosphate buffer, pH 7.4, and 0.1 mM 5-[2-^{14}C]-serotonin binoxalate (NEN specific activity 48.3 mCi/mmol), or β-[ethyl-1-^{14}C]-phenylethylamine hydrochloride (NEN specific activity 50.0 mCi/mmol), as a substrate in 300 μl total volume. The reaction was terminated by the addition of 0.2 ml 2 N HCl and the acidic reaction product extracted into 5 ml of toluene for counting.

Muscarinic cholinergic receptors were measured by the specific ^{3}H-quinuclidinyl benzilate (QNB) binding assay described by Yamamura *et al.* [51]. A portion of homogenate was suspended in an appropriate volume of 50 mM phosphate buffer pH 7.4 and was centrifuged at 48,000 × g for 10 minutes. Pellets were washed by resuspending twice in the buffer and recentrifuging. The membrane fraction obtained was incubated for 60 min at 25°C in 0.05 M phosphate buffer pH 7.4 with 0.6 nM ^{3}H-QNB (NEN specific activity 40.2 Ci/mmol) in a final volume of 2.0 ml. Specific binding was defined as the difference in total ^{3}H-QNB bound in the absence and presence of 1 μM unlabelled QNB. The binding reaction was terminated by vacuum filtration through Whatman GF/B filters, the filters were washed three times with 5 ml of the phosphate buffer and the radioactivity on the filter was determined.

The adrenergic receptor binding assay was performed by the method of Bylund and Snyder [7]. Tissue homogenates were suspended in 50 mM Tris-HCl buffer pH 8.0 and centrifuged at 48,000 × g for 15 min. The resulting pellets were washed once in the same buffer and centrifuged. The membrane fraction obtained was resuspended in 50 mM Tris-HCl buffer, pH 8.0, containing 0.1% ascorbic acid and 1 μM pargyline. The membrane suspensions were incubated with 1 nM of ^{3}H-dihydroalprenolol (DHA) (NEN specific activity 44.9 Ci/mmol) for 20 minutes at 24°C. The binding reaction was terminated by rapid vacuum filtration through Whatman GF/B filters and the filters were washed three times with 5 ml

of cold buffer. The radioactivity on the filers was counted. The specific binding was determined by subtracting nonspecific binding measured in the presence of 1 μM alprenolol from total binding.

The specific binding of ^{3}H-spiroperidol to cortical membranes was measured by the method of Creese *et al.* [8]. Tissue homogenate was suspended in 50 mM Tris-HCl buffer, pH 7.7, centrifuged at 48,000 × g, resuspended in fresh buffer and recentrifuged. The final pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.1, containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. The sample was incubated with 0.15 nM ^{3}H-spiroperidol (Amersham specific activity 25 Ci/mmol) for 20 minutes at 37°C and rapidly filtered under vacuum through Whatman GF/B filters followed by rapid washing with three 5 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.7. The specific binding was obtained by subtracting nonspecific binding measured in the presence of 1 mM dopamine from the total binding.

RESULTS

The weight at sacrifice of the rats averaged 569±64(S.D.) for the deficient group and 831±89 for the control group, the highly significant difference being consistent with the chronic deficiency.

The levels of lipid peroxides in various brain areas show only small regional variations (Table 1). In four month old rats, the levels ranged from 202 nmole/g protein in spinal cord to 335 nmole/g protein in the substantia nigra. In eighteen month old control rats, the lipid peroxide levels were increased overall by split plot factor analysis and particularly significant increases were observed in the olfactory bulb, globus pallidus, cerebral cortex and caudate-putamen.

In vitamin E-deficient as compared to control rats of the same age, lipid peroxides were significantly increased in the cerebral cortex ($p<0.001$), hippocampus ($p<0.05$) and hypothalamus ($p<0.05$) (Table 1).

Some of the data from the various neurotransmitter-related enzyme assays and binding studies are given in Table 2. It should be noted that not all assays were done on all brain regions because of tissue scarcity. In addition to the results shown, AChE was determined in the substantia nigra and cerebellum, GAD in the cortex, caudate-putamen and globus pallidus, tyrosine hydroxylase in the caudate-putamen and spiroperidol binding in the cortex but the detailed data are omitted because no significant effect of age or vitamin E deficiency was noted.

The only age-related decreases found were in ChAT, AChE and ^{3}H-QNB binding and, in each case, significant changes were found only in some of the regions examined. MAO, measured with either substrate, and GABA-T, on the other hand, showed significant age-related increases in all of the few areas in which they were studied.

In comparison with control rats of comparable age, the vitamin E-deficient group showed no significant increases and significant decreases only in ChAT in the substantia nigra (of 23%) and cerebellum (12%), of ^{3}H-QNB binding in the caudate-putamen (33%), and of ^{3}H-DHA binding in the cortex (38%).

DISCUSSION

The present studies have demonstrated an age-associated increase of lipid peroxides in four out of ten rat brain regions studied (Table 1). The increase was enhanced significantly in the cortex by feeding the rats for nine months prior to death with a vitamin E-deficient diet, but no enhancement was seen in the olfactory bulb, globus pallidus and caudate-putamen. On the other hand, significant increases in the vitamin E-deficient group as compared with controls were found in the hippocampus and hypothalamus where the effect of age was not significant.

Various kinds of reactive oxygen species are generated constantly by enzymic and non-enzymic processes in biological systems. Tissue damage due to free radical formation has been believed to be an important factor in aging and age-associated diseases [9, 12, 13, 21]. The central nervous system (CNS) is though to be particularly susceptible to the attack of active oxygen species because neurons are abundant in the unsaturated fatty acids and phospholipids which are the main targets of peroxidation. In addition, the metabolic rate of brain tissue is extremely high and neurons are postmitotic cells. Lipofuscin, which may be formed by polymerization of oxidized polyunsaturated fatty acids, accumulates with age mainly in these postmitotic cells. It has been reported that vitamin E deficiency increases CNS lipofuscin deposits and depresses CNS function. The most significant chemical properties of vitamin E are considered to be its functions as a free radical scavenger and as a quencher of singlet oxygen [9, 21, 26, 36]. It may protect membranes by preventing excessive lipid peroxidation and subsequent cell damage in biological systems [21]. We previously observed a significant increase of lipid peroxides in the brains of young rats after hyperbaric oxygen exposure. The elevation of lipid peroxides was transient, however, and returned very quickly to preexposure levels. Even after repeated hyperbaric oxygen exposure, there was no lasting accumulation of lipid peroxides [31]. These observations suggest that excess lipid peroxides, which are extremely harmful for neuronal cells, are quickly metabolized and thus kept at low levels in young animals. It remains to be shown whether the higher levels observed in old animals reflect an increased rate of free radical generation, the reduction of

TABLE 1

EFFECT OF AGE OR VITAMIN E DEFICIENCY ON LIPID PEROXIDES IN VARIOUS REGIONS OF RAT BRAIN

	Controls			Vitamin E Deficiency (7)
	4 month (8)		18 month (6)	
Olfactory bulb	285 ± 45	*	415 ± 36	430 ± 94
Globus pallidus	290 ± 24	*	378 ± 25	380 ± 16
Caudate-putamen	254 ± 21	†	289 ± 26	327 ± 17
Cerebral cortex	241 ± 24	*	314 ± 28	† 591 ± 191
Hippocampus	257 ± 28		281 ± 40	§ 343 ± 56
Thalamus	289 ± 28		316 ± 48	343 ± 51
Hypothalamus	256 ± 36		288 ± 26	§ 358 ± 62
Substantia nigra	335 ± 55		354 ± 48	360 ± 42
Cerebellum	210 ± 42		224 ± 22	262 ± 52
Spinal cord	202 ± 33		277 ± 87	284 ± 59

Lipid peroxides are expressed as nmol MDA per g protein. Each value represents mean±SD from the number of rats expressed in parentheses. Significant differences between pairs by the Student's *t*-test are indicated by *$p<0.001$; †$p<0.01$; ‡$p<0.02$; and §$p<0.05$.

TABLE 2

EFFECT OF AGE OR VITAMIN E DEFICIENCY ON VARIOUS NEUROTRANSMITTER-RELATED ENZYMES AND BINDING SITES*

	Controls			Vitamin E Deficiency (7)
	4 month (8)		18 month (6)	
ChAT				
Cortex	45.6 ± 3.1		44.1 ± 4.1	45.7 ± 5.4
Caudate-putamen (CP)	172.7 ± 12.3	*	127.5 ± 8.3	134.0 ± 8.5
Globus pallidus	28.4 ± 2.5	*	21.1 ± 3.8	17.3 ± 2.8
Hippocampus	43.3 ± 2.9	*	32.1 ± 2.5	32.9 ± 1.6
Substantia Nigra	6.8 ± 0.7		7.3 ± 1.0	† 5.6 ± 1.6
Cerebellum	9.6 ± 1.9		9.1 ± 0.6	‡ 8.0 ± 0.8
AChE				
Cortex	78.5 ± 4.7		76.0 ± 6.9	80.0 ± 8.4
Caudate-putamen	302.3 ± 15.2	*	225.7 ± 10.8	233.5 ± 10.8
Hippocampus	61.1 ± 2.5	†	53.8 ± 5.2	53.7 ± 4.6
GABA-T				
Cortex	300 ± 11	†	328 ± 19	341 ± 6
Caudate-putamen	340 ± 34	†	408 ± 29	420 ± 35
Globus pallidus	272 ± 34	*	354 ± 14	357 ± 38
"MAO A" in CP	38.9 ± 3.1	*	44.5 ± 1.7	43.2 ± 4.0
"MAO B" in CP	29.2 ± 1.3	*	31.8 ± 1.3	30.6 ± 1.6
QNB Binding				
Cortex	1009 ± 82	†	831 ± 95	857 ± 49
Caudate-putamen	1204 ± 107		1255 ± 194	† 846 ± 228
Hippocampus	966 ± 145		972 ± 51	926 ± 58
^{3}H-Dihydroalprenolol Binding				
Cerebral cortex	33.0 ± 4.5		29.0 ± 4.6	* 18.1 ± 1.9

Each value represents mean±S.D. from the number of rats shown in parentheses ChAT, GABA-T, and MAO activities are in nmol/mg protein/hr; AChe in nmol/mg protein/min; and the binding data are in fmol/mg protein. Significant differences between pairs by the Student's t-test are indicated by *$p<0.001$; †$p<0.01$; ‡$p<0.02$; and §$p<0.05$.

defensive mechanisms against lipid peroxidation, or both. If steady state lipid peroxidation is abnormally increased and uncontrolled as a result of aging processes, the high levels may cause irreversible damage to neuronal cells.

The present data on neurotransmitter-related enzymes and binding sites in the vitamin E-deficient animals do not lend much support to this hypothesis since the changes seen in this group as compared with age-matched controls were few in number, rather small in magnitude and generally correlated poorly with changes in lipid peroxides. In Table 3 it can be seen that the only age-related changes in neurotransmitter related indices which might correlate across various regions with age-related changes in lipid peroxide levels are those in GABA-T and MAO A and B. The MAO(s) were, however, only measured in the caudate-putamen and GABA-T did not show, in the cortex of vitamin E deficient animals, the additional increase found in lipid peroxide levels (Table 1 and 2).

The age-related changes in neurotransmitter systems indicated by the present data are only partly consistent with previous literature. One reason may be that the "aged" rats used in this study are considerably younger than the 22–26 month old animals used in most investigations on age-related effects.

Both MAO A and B were increased slightly in the single area (the caudate-putamen) where they were measured. There have been a number of studies on MAO in aged animals and humans with many, but not all, investigators reporting some increase with age. The reference most pertinent to the present data is probably that of Strolin Benedetti and Keane [39] who compared 2 and 24 month old male Sprague-Dawley rats and found that MAO A activity (assayed with serotonin as substrate) decreased by about 19% with age in the striatum while MAO B activity (assayed with β-phenylethylamine as substrate) increased by about 17% in the same region. Our result on MAO B is consistent with this report but it is inconsistent that we also found an increase in MAO A and in the MAO A to MAO B ratio.

We know of no previous report on the effects of aging on GABA-T but, since this is at least partly a glial enzyme [4], it is not too surprising that, like MAO B, it should increase with age since the proportion of glia presumably increase.

The only decreases with age found in the present study were in indices related to the cholinergic system. The losses in ChAT and AChE with age are not surprising in view of available literature except that they are rather larger than might be expected and that no significant decrease was seen in the cerebral cortex. A decline in ChAT activity with aging has been reported in rodent cerebral cortex [41] and rat neostriatum [22, 25, 41], while both significant [47] or non-significant [41,42] decreases in ChAT have been reported in rodent hippocampus. The small but significant decline in rat

TABLE 3

SUMMARY OF SIGNIFICANT DIFFERENCES FOUND IN LIPID PEROXIDE LEVELS AND IN VARIOUS NEUROTRANSMITTER-RELATED ENZYMES AND BINDING SITES BETWEEN 4 AND 18 MONTH OLD CONTROLS*

Area	Lipid Peroxides	ChAT	AChE	GABA-T	"MAO A"	"MAO B"	^{3}H-QNB Binding
Cerebral cortex	In 23%	—	—	In 9%	N.D.	N.D.	De 18%
Caudate-putamen	In 12%	De 26%	De 25%	In 20%	In 14%	In 9%	—
Globus pallidus	In 23%	De 25%	N.D.	In 30%	N.D.	N.D.	N.D.
Hippocampus	—	De 26%	De 12%	N.D.	N.D.	N.D.	—

*No significant age-related differences were found in the substantia nigra or cerebellum. The assay for lipid peroxide levels was the only one done in the olfactory bulb, thalamus and spinal cord and only in the first was there a significant effect of age (Table 1). De=decreased in test as compared to control group; In=increased; —=no significant difference found; and N.D.=not determined.

neostriatal ChAT reported by some [22, 25, 41] was not confirmed by Reis *et al.* [37], and Strong and Gottesfeld [40] suggest the decline in ChAT with age (30–50% decrease in 26 month old compared to 6 month old rats) may be different in different parts of the striatum.

In rats from 3–24 months of age, decrements have also been reported in AChE in the forebrain (17%) and cerebral cortex (10–25%) [14]. In previous studies, using a different assay for AChE, we were unable to find a significant effect of age on neostriatal AChE [22] and similar negative results have been reported by Meek *et al.* [25]. In view of the different forms of AChE which exist in brain, much may depend upon the exact assay conditions.

The literature on changes in muscarinic binding sites with aging in rats is also somewhat contradictory. Misra *et al.* [27] found a loss of muscarinic binding sites with age in rat hippocampus and Lippa *et al.* [18] report both physiological and binding data indicating about a 20% decrease in the dorsal hippocampus of 26–28 month as compared to 6–8 month old rats; such a loss was not confirmed by Strong *et al.* [41], although they found significant decrements in cortex and striatum. Morin and Wasterlain [28], like ourselves, found a substantial decrease with age in the caudate-putamen but not in the cortex or hippocampus; much may depend upon the particular regions of these relatively large structures.

No decrease was found with age in any of the catecholamine indices measured. Significant and sometimes very large decreases in striatal TH have been repeatedly reported in 22–27 month old rats [2, 3, 15, 22, 24, 37], but the data suggest the declines only occur at very advanced ages. Hence it is not too surprising that no significant change was seen in the 18 month old animals used here.

The failure to find changes in ^{3}H-spiroperidol or ^{3}H-DHA-binding in the cerebral cortex of 18 month as compared to 4 month old rats is not surprising in view of the available literature. Fairly large decreases with age in rats in the binding of various labeled dopamine antagonists have been reported by a number of laboratories for regions such as the striatum and olfactory tubercle, but no such declines have been reported in the cortex (where ^{3}H-spiroperidol may bind to serotonin as well as dopamine recognition sites). Although some [27,49] have reported age-related losses of β-adrenergic binding sites in rat cerebral cortex, others [20,34] have not found such decreases. Since all of the binding assays were done at only one concentration, any changes in affinity would complicate the interpretation but most reported age- (and drug-) related changes in binding seem to be in numbers rather than the affinities of the binding sites.

There are, of course, many other references suggesting changes in these neurotransmitter indices in aging [23,35] but we have limited the discussion here to those dealing with the rat brain regions we have studied since there are clearly marked species and regional variations in the effects noted.

Further work is planned to see if these or other neurotransmitter indices are more affected by more prolonged vitamin E deficiency or by repeated exposure to hypobaric oxygen.

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