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Changes in Cholinergic Activity in Human Hippocampus Following Chronic Alcohol Abuse

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NORDBERG, A., C. LARSSON, E. PERDAHL AND B. WINBLAD. *Changes in cholinergic activity in human hippocampus following chronic alcohol abuse.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 397–400, 1983.—Cholinergic mechanisms in the hippocampus seem to play a role in memory function. Since it is well known that chronic alcoholics often have a disturbed memory function, the cholinergic activity of the hippocampus has been measured in 20 chronic alcoholics and 14 controls, post-mortem. Of the alcoholics, 13 were classified as "intoxicated" alcoholics and 7 as "sober" alcoholics, i.e., without ethanol in blood or urine at the time of death. A lower, although not significantly lower activity of the enzyme choline acetyltransferase (ChAT, presynaptic marker) was measured in the hippocampus of the chronic alcoholics as compared to the control group. A trend towards a lower number of muscarine-like binding sites was also observed for the alcoholic group, but was only significant for the oldest group of alcoholics (59–68 years; −30%; $p<0.01$) in comparison to controls. No significant change in the number of nicotine-like binding sites was found. A normal aging process with degenerative nerve cell changes might, in combination with an excessive intake of ethanol, lead to the more pronounced decrease in muscarine-like binding sites found for the older alcoholics.

Chronic alcoholism Human brain Hippocampus Choline acetyltransferase Muscarine-like receptors Nicotine-like receptors

THERE is evidence to indicate that cholinergic mechanisms and the hippocampus play a role in memory function [4]. Since chronic alcoholics have deficient memory function, we were interested in studying possible changes in cholinergic parameters. In two earlier preliminary human post-morten brain studies, a reduced number of muscarine-like binding sites was found [1,9]. In addition choline acetyltransferase (ChAT) activity in the hippocampus and cerebellum was reduced in the group of chronic alcoholics [1]. The concentration of brain monoamines in post-mortem tissue from chronic alcoholics has also been reported to be reduced to a similar level as that seen in senile dementia [3]. The aim of the present study was to further investigate cholinergic activity in the hippocampus in a group of chronic alcoholics.

METHOD

Twenty individuals were classified as chronic alcoholics due to hospital, police and social records, histopathology of the liver, as well as analysis of ethanol in blood and urine. Fourteen normal individuals comprised a control group (Table 1). The chronic alcoholics had a long- well-documented history of alcohol abuse, and at autopsy a liver steatosis or cirrhosis was found. Of the chronic alcoholics, 13 were classified as "intoxicated" alcoholics and 7 as "sober" alcoholics, i.e. without ethanol in blood or urine at the time of death (Table 1). The controls, who had no previous history of alcohol abuse, died acutely of heart infarction or accident. Both alcoholics and controls were autopsied at the Forensic Department, Umeå University. The corpses were kept at +5°C until autopsy. As seen in Table 1, there was no significant difference in time from death to autopsy between alcoholic and control groups. After removal of the brain, the complete hippocampus was cut out, after the fimbriae had been removed. The hippocampus was kept frozen at −80°C until analysis. The activity of choline acetyltransferase (ChAT) was measured by a radioenzymatic method using ^{14}C-labelled acetylcoenzyme A [2,5]. Cholinergic receptor binding sites were measured after preparation of a crude synaptosomal fraction (P2 fraction). The muscarine-like binding sites were quantitated by using the labelled muscarinic antagonist quinuclidinyl benzilate ((−) ^{3}H-QNB; 43 Ci/mmol; 0.2 nM) as ligand [11]. Correction for nonspecific binding was made by parallel experiments including 10^{-4} M atropine. For the nicotine-like binding sites two labelled antagonists were used: tubocurarine (^{3}H-TC; 15.8 Ci/mmol; 3 nM) and α-bungarotoxin (^{3}H-Btx; 61 Ci/mmol; 1.5 nM). The specific binding of these ligands was calculated by subtracting the values for non-specific binding in presence of 10^{-4} M unlabelled tubocurarine from the values for total

TABLE 1
SOME BASIC DATA

	Controls n=14	Total Chronic alcoholics n=20	Chronic alcoholics Intoxicated n=13	Chronic alcoholics Sober n=7
Age (yrs)	55 ± 3 (range 31–68)	47 ± 3 (range 29–65)	45 ± 4	51 ± 3
Sex	12♂ 2♀	16♂ 4♀	10♂ 3♀	6♂ 1♀
Time from death-autopsy (hr)	56 ± 7	58 ± 7	57 ± 10	59 ± 9
Brain weight (g)	1441 ± 25	1465 ± 33	1481 ± 32	1436 ± 76
Blood chemical ‰	—	range 0.9–4.7	range 0.9–4.7	—

Mv ± SE
Values represent mean ± SEM.
n=number of individuals

binding (for further details, see [7]). The protein content of the P2 fraction was measured [8] and the amount of radioactive ligand bound to the P2 fraction was expressed in pmol/g protein.

RESULT AND DISCUSSION

In this study the activity of ChAT and the number of cholinergic receptor binding sites have been measured in hippocampal tissue from each individual in the chronic alcoholic and control group. ChAT is the catalysing enzyme in the synthesis of ACh and can therefore be considered as a presynaptic marker, while the receptor binding sites are mostly considered to be postsynaptic. As seen in Table 2 there was a lower activity of ChAT in the hippocampus of the chronic alcoholics as compared to the controls, although this difference was not statistically significant. The lowest ChAT activity was found in the group of "sober" alcoholics. A trend towards a lower number of muscarine-like binding sites was also noticed in the chronic alcoholics. No difference was observed in nicotine-like binding sites between the groups. When the data obtained from the chronic alcoholics was analyzed according to the age of the individuals, (Table 3) a significantly lower number of muscarine-like binding sites was obtained in the oldest group of alcoholics (age range 59–68 years) (−30%; $p<0.01$) in comparison to controls. In this age group a tendency towards a lower number of nicotine-like binding sites (^{3}H-TC, −19%; ^{3}H-Btx, −38%) was also seen. No significant difference in muscarine- and nicotine-like binding sites could be observed between "intoxicated" and "sober" alcoholics. In the oldest group of alcoholics, in whom a significant decrease in muscarine-like binding sites was found, the small number of investigated subjects did not allow any valid separation into "sober" and "intoxicated" alcoholics.

The decrease in muscarine-like binding sites in the hippocampus of chronic alcoholics which was found in this study was less marked than that found in our previous study [9]. However, in that study the control material was obtained from hospitalized patients taken directly to the hospital pathology department. Since a considerable problem involved in post-mortem studies of human brain from a Forensic Department is the long time-lapse between death and autopsy, it is of utmost importance that the control and experimental tissue be taken under identical conditions, as was done in this study.

It is interesting to observe that when the number of muscarine-like binding sites is plotted against ChAT activity in the hippocampus of each individual a positive correlation ($p<0.05$; $r=0.49$) is found in the group of chronic alcoholics (Fig. 1). The lowest measured number of muscarine-like binding sites (62 pmol/g protein) was found in a 52 year old "sober" alcoholic who had a long history of ethanol abuse and a periodical methanol abuse. The ChAT activity in the hippocampus from this individual was also the lowest measured in the total material (6 pkatal/g tissue). Since chronic alcoholism and senile dementia both are associated with memory disorders in which cholinergic mechanisms seem to be involved, it is interesting to note that in senile dementia of Alzheimer type (SDAT) a negative correlation between ChAT activity and muscarine-like binding sites has been found, suggesting a receptor compensation [10]. Thus, while the primary lesion in SDAT might be at the presynaptic site of the cholinergic nerve terminal the findings in chronic alcoholics indicate a more general effect of ethanol on the cholinergic nerve terminal.

A neuronal loss in the hippocampus after prolonged ethanol treatment has been reported [14]. It might also be possible that ethanol and/or its metabolite acetaldehyde, can be directly neurotoxic, or exert their effects by inhibiting protein synthesis [13] or by altering cerebral blood flow, resulting in ischemia [6]. A decrease in hippocampal muscarine-like binding sites has been measured during normal aging [12]. Such a change in combination with degenerative nerve cell changes and losses induced by ethanol, might result in an increased vulnerability and consequently in a more pronounced decrease of muscarine-like binding sites in older chronic alcoholics.

TABLE 2

ChAT ACTIVITY, MUSCARINE- AND NICOTINE-LIKE RECEPTOR BINDING SITES IN HIPPOCAMPUS OF CHRONIC ALCOHOLICS AND CONTROLS

	Controls n=14	Chronic alcoholics n=20	"Intoxicated" n=13	"Sober" n=7
ChAT (pkatal/g tissue)	59 ± 14	41 ± 5	48 ± 7	29 ± 8
QNB (pmol/g protein)	334 ± 17	298 ± 18	303 ± 14	287 ± 46
TC (pmol/g protein)	91 ± 10	88 ± 4	83 ± 5	95 ± 7
Btx (pmol/g) protein)	7 ± 1	6 ± 1	6 ± 1	7 ± 2

The group of chronic alcoholics was separated into two groups "intoxicated" and "sober" alcoholics (for further details see Method section).
n=Number of individuals.
Values represent mean ± SEM.

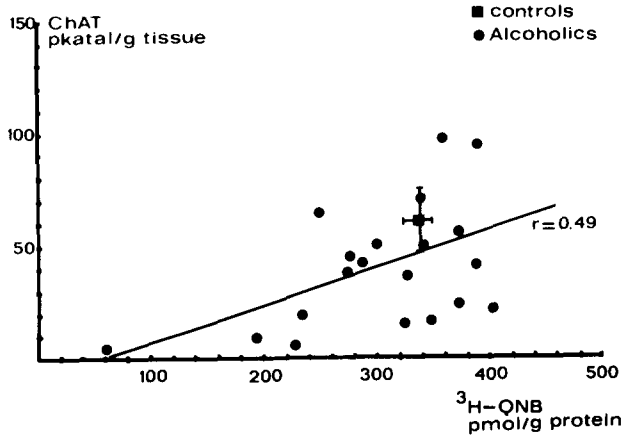

FIG. 1. Relationship between number of muscarine-like binding sites and ChAT activity in the hippocampus of chronic alcoholics. Mean value of the control group is also given, together with relevant standard errors.

TABLE 3

NUMBER OF MUSCARINE-LIKE BINDING SITES IN HIPPOCAMPUS OF CHRONIC ALCOHOLICS AND CONTROLS AT DIFFERENT AGES

	³H-QNB binding sites (pmol/g protein)	
Age (yrs)	Controls	Chronic alcoholics
29–30	318 n=2	307 ± 18 n=7
39–48	320 n=2	337 n=2
49–58	303 ± 28 n=5	299 ± 54 n=6
59–68	387 ± 23 n=5	271 ± 21* n=5

Values represent mean ± SEM. *$p<0.01$, compared to controls.

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REFERENCES

1. Antuono, P., S. Sorbi, L. Bracco, T. Fusco and L. Amaducci. A discrete sampling technique in senile dementia of the Alzheimer type and alcoholic dementia: study of the cholinergic system. In: *Aging of the Brain and Dementia, Aging*, vol. 13, edited by L. Amaducci, A. N. Davison and P. Antuono. New York: Raven Press, 1980, pp. 151–158.

2. Aquilonius, S. M., S. Å. Eckernäs and P. G. Gillberg. Topographical localization of choline acetyltransferase within the human spinal cord and a comparison with some other species. *Brain Res* **211:** 329–340, 1981.

3. Carlsson, A., R. Adolfsson, S. M. Aquilonius, G. C. Gottfries, L. Oreland, L. Svennerholm and B. Winblad. Biogenic amines in human brain in normal aging, senile dementia and chronic alcoholism. In: *Ergot Compounds and Brain Function: Neuroendocrine and Neuropsychiatric Aspects*, edited by M. Goldstein, A. Lieberman, D. B. Calne and M. O. Thorner. New York: Raven Press, 1979, pp. 295–304.
4. Drachman, D. A. Memory, dementia and the cholinergic system. In: *Alzheimer Disease: Senile Dementia and Related Disorders*, edited by R. Katzman, R. D. Terry and K. L. Blick. New York: Raven Press, 1978, pp. 141–148.
5. Fonnum, F. A rapid radiochemical method for the determination of choline acetyltransferase. *J Neurochem* **24:** 407–409, 1975.
6. Goldman, H., L. A. Sapirstein, S. Murphy and J. Moore. Alcohol and regional blood flow in brain of rats. *Proc Soc Exp Biol Med* **144:** 983–988, 1973.
7. Larsson, C. and A. Nordberg. Studies of nicotine-like binding sites in brain. In: *Neurotransmitters and Their Receptors*, edited by U. Z. Littauer, I. Silman, V. I. Teichberg and Z. Vogel. New York: John Wiley and Sons, 1980, pp. 297–301.
8. Lowry, O. H., N. J. Rosenbrough, A. L. Farrand, R. J. Randall. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
9. Nordberg, A., R. Adolfsson, S. M. Aquilonius, S. Marklund, L. Oreland and B. Winblad. Brain enzymes and acetylcholine receptors in dementia of Alzheimer type and chronic alcohol abuse. In: *Aging of the Brain and Dementia, Aging*, vol. 13, edited by L. Amaducci, A. N. Davison and P. Antuono. New York: Raven Press, 1980, pp. 169–172.
10. Nordberg, A., C. Larsson, R. Adolfsson, I. Alafuzoff and B. Winblad. Muscarinic receptor compensation in hippocampus of Alzheimer patients. *J Neural Transm*, in press, 1982.
11. Nordberg, A., G. Wahlström and C. Larsson. Increased number of muscarinic binding sites in brain following chronic barbiturate treatment to rat. *Life Sci* **26:** 231–237, 1980.
12. Nordberg, A. and B. Winblad. Cholinergic receptors in human hippocampus-regional distribution and variance with age. *Life Sci* **29:** 1937–1944, 1981.
13. Tewari, S., S. Murray and E. P. Noble. Studies on the effects of chronic ethanol ingestion on the properties of rat brain ribosomes. *J Neurosci Res* **3:** 375–387, 1978.
14. Walker, D. W., E. E. Barnes, S. F. Zormetzer, B. E. Hunter and P. Kubanis. Neuronal loss in hippocampus induced by prolonged ethanol consumption in rats. *Science* **209:** 711–712, 1980.