THE CHOLINERGIC SYSTEM AND MEMORY: AMELIORATION OF ETHANOL-INDUCED MEMORY DEFICIENCY BY PHYSOSTIGMINE IN RAT

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Abstract. Male Sprague-Dawley rats were treated with a solution of ethanol (20% v/v) as the only source of fluid for 28 weeks while controls received tap water. Profound reductions of acetylcholine content, the activities of choline acetyltransferase and acetylcholinesterase and choline uptake were observed in the cortex, hippocampus, substantia innominata and striatum four weeks after withdrawal from ethanol. These changes in cholinergic markers were accompanied by an impaired performance in a spontaneous alternation task tested at long acquisition-retention intervals. This ethanol induced behavioural deficiency, which most likely represents a memory impairment, could be reversed by application of physostigmine (0.45 g/kg; i.p.) whereas neostigmine did not show any behavioural effect. The results suggest that ethanol-induced memory impairment can be ameliorated by pharmacological manipulation of central cholinergic function.

INTRODUCTION

Chronic consumption of ethanol is known to cause brain damage accompanied by an amnesic or dementing syndrome (37). Deficiencies in the cholinergic innervation of the cortical mantle as a result of neu-
ronal damage in the basal forebrain projection system in chronic alcoholics has been demonstrated by means of biochemical (1, 12, 31-33) and quantitative morphological investigations (4, 5). In previous experimental studies of prolonged ethanol intake in rat, we demonstrated a progressive decline in memory function, which was paralleled by a decrease in the levels of presynaptic cholinergic markers in the basal forebrain projection system and in other brain areas (2, 7). Neuronal loss at the site of origin (39) of the cholinergic innervation of the cortical mantle in the basal forebrain, which can be induced by chronic ethanol intake in rat (6) and mice (9), shows a quantitative relationship to the degree of cognitive dysfunction (5). Furthermore, a pathogenetic link between ethanol-induced memory deficiency and impairment of the cholinergic afferentation of the cortical mantle is supported by behavioural sequelae of grafting of cholinergic-rich foetal basal forebrain cell suspensions to cortex and hippocampus (2, 3). Besides postalcoholic Korsakoff’s syndrome, damage of the cholinergic basal forebrain projection system has been observed in a variety of other neuropsychiatric disorders which are associated with amnesia or dementia such as Alzheimer's disease or Parkinson's disease (4, 10). Postalcoholic Korsakoff’s syndrome shares not only this neurobiological substrate with these neurodegenerative disorders. Similarities in the pattern of neuropsychological dysfunctions in Korsakoff’s psychosis, Alzheimer’s and Parkinson’s disease have repeatedly been demonstrated as well (11, 23-25, 27, 30). The brain-behaviour-relationship under these conditions might, therefore, be described by means of a 'syndrome of partial cholinergic deafferentation of the cortical mantle' (5). Brain damage after chronic intake of ethanol, however, is more widespread and not restricted to central cholinergic systems (2).

In order to further test the pathogenetic significance of cholinergic dysfunction for the development of ethanol induced cognitive impairment, the behavioural effects of central acetylcholinesterase inhibition by application of physostigmine was investigated in rat after chronic intake of ethanol in the present study.

MATERIAL AND METHODS

**Ethanol treatment**

Male Sprague-Dawley rats (160-180 g) were caged individually and maintained under artificial 14-10 h light/dark cycle. Ethanol was administered in the drinking water (20% v/v) as the only source of fluid for 28 weeks while control animals received tap water. Free access to food pellets was provided throughout the experiment. Body weight and
fluid intake was regularly recorded. During the final week of ethanol treatment, blood was withdrawn from the tail in both the morning (10 a.m.) and the evening (11 p.m.). Animals were slowly withdrawn from ethanol intake over 9 days and then maintained on water for another 4 weeks before behavioural testing was commenced. No seizures were noted during withdrawal.

**Behavioural testing**

Animals were tested in a closed T-maze on a spontaneous alternation task (8). The stem and each arm of the maze were 50 cm long and 10 cm wide surrounded by a wall of a height of 25 cm. Rats received two forced acquisition trials followed by a free retention trial with varying acquisition-retention intervals. During acquisition animals were forced to enter one of the arms twice, the other on being blocked. On the test for retention, animals had free access to both arms. Animals were randomly allocated to one of three different groups, receiving the retention trial 30 s, 5 min or 1 h after completion of the forced acquisition trials.

**Pharmacological treatment**

Three days after having received two acquisition and one retention trials, animals were tested under the influence of physostigmine (P), neostigmine (N) or saline (S) injections in the same behavioural paradigm. Animals were tested twice for each retention interval (30 s, 5 min, 1 h) under each drug condition. Thus, each animal received 18 consecutive tests which were separated by two days, the sequence being P, N, S, ...; N, P, S, ... or S, P, N, ... i.p. injections of physostigmine sulphate (0.45 mg/kg), neostigmine methylsulphate (0.45 mg/kg) or equivalent volumes of saline were given 20 minutes prior to the two acquisition trials and the retention trial respectively.

**Biochemical assays**

Animals were killed by decapitation (for determination of enzyme activities and choline uptake) or by focused microwave radiation (determination of acetylcholine content). Cortex, hippocampus, striatum and substantia innominata were dissected out, weighed and homogenized in ice-cold 0.25 M sucrose containing 0.2% Triton X-100 (enzymes), in 0.36 M sucrose (choline uptake) or in trichloroacetic acid (acetylcholine).

Choline acetyltransferase (ChAT, EC 2.3.1.6) was measured according to Fonnum (19), acetylcholinesterase (AChE, EC 3.1.1.7) according to Ellman et al. (16). [3H]Ch uptake was determined as described by Marchbanks et al. (28) on Percoll gradients (15). ACh was assayed by the chemiluminescent method of Israel and Lesbats (21). Protein content was
measured according to Peterson (34). Blood alcohol was determined with a Lion Alcometer (AE-D1).

RESULTS

Body weight and ethanol intake

Increase in body weight of ethanol-treated animals was slightly less than that of tap water controls (Fig. 1). At the end of treatment and at the time of behavioural testing, however, differences were not statistically significant (Student's t-test; $p > 0.1$). Mean daily ethanol consumption varied between 12.2 g/kg of body weight in the beginning and 9.7 g/kg at the end of ethanol treatment (Fig. 1) which resulted in blood alcohol levels between 40 (daytime) and 120 mg/100 ml (darkness).

![Graph showing changes in body weight and ethanol intake over 35 weeks.](image)

**Fig. 1.** Changes in body weight of non-ethanol-treated controls and ethanol treated rats over 28 weeks of treatment and during and after withdrawal from ethanol. Data are mean (+SEM) of 30 controls (filled circles) and 30 experimental animals (open circles). Ethanol-treated animals tended to weight less than the controls, differences, however, were not significant. Mean daily ethanol consumption of treated animals is indicated by a dashed line (filled triangles).

Biochemical analysis

Reduction in ACh content, in the activities of ChAT and AChE and in Ch-uptake in cortex, hippocampus, substantia innominata and striatum after ethanol treatment are shown in Fig. 2. The changes were most
Fig. 2. Changes in the content of ACh, in the activities of ChAT and AChE and in Ch-uptake in the cortex, hippocampus, substantia innominata and striatum after a 4 weeks ethanol-free period following 28 weeks of ethanol intake. Data are expressed as percentages of the following control values of no-ethanol treated animals (bars: SEM): ACh (pmol/mg wet weight; n = 5); cortex (c) 25.7 +/− 0.5; hippocampus (h) 28.8 +/− 0.9; subst. innom. (i); 37.0 +/− 1.3; striatum (s); 67.2+/−2.6 ChAT (nmol/mg protein/hr; n = 5); c, 47.7+/−1.1; h, 79.6+/−1.8; i, 153.8+/−2.3; s, 181.4+/−3.1; AChE (μmol/mg protein/hr; n = 5); c, 4.1+/−0.1; h, 7.3+/−0.5; i, 14.6+/−0.9; s, 17.2+/−1.8; Ch-uptake (pmol/mg protein/min; n = 5); c, 8.1+/−0.3; h, 13.4+/−0.6; i, 23.5+/−1.2; s, 27.7+/−1.8. All changes are significant (Student's t-test, *p < 0.05; **p < 0.01; ***p < 0.001).

pronounced in the substantia innominata where the levels of these markers decreased to 60%/−45% of control values whereas cortex, hippocampus and striatum showed reductions to about 80%/−50%. In all four regions ACh content was more severely affected than the activities of ChAT and AChE or Ch-uptake.

**Behaviour**

The effect of ethanol treatment on the performance of the spontaneous alternation task is shown in Fig. 3. Whereas on short retention intervals ethanol-treated animals performed as well as non-treated animals, a significant impairment (ANOVA, p < 0.001) could be demonstrated for a retention interval of one hour where animals performed near to chance level.
Fig. 3. Rate of spontaneous alternation of ethanol-treated animals and controls as a function of the duration of the retention interval. Data are mean of 10 animals +/-SEM. Experimental animals are significantly impaired for a retention interval of one hour (Student's t-test, p < 0.001).

Fig. 4. Rate of spontaneous alternation of water controls and ethanol-pretreated animals tested under the influence of physostigmine, neostigmine and saline. Data are mean of 10 animals (bar: SEM). Drugs were given 20 min before acquisition and retention trials (0.45 g/kg i.p.). Physostigmine significantly improved performance of ethanol-treated animals (Student's t-test, p < 0.001).
**Behavioural results of pharmacological manipulation of ethanol pre-treated animals by physostigmine or neostigmine are shown in Fig. 4. Performance was similarly impaired under neostigmine or without pharmacological treatment (ANOVA, vs. water controls, p < 0.001). Physostigmine, however, significantly improved the performance (ANOVA, ethanol + physostigmine vs. ethanol, p < 0.001) almost reversing it to control values. No behavioural effects of pharmacological treatments could be demonstrated for control animals.**

**DISCUSSION**

The results of the present study demonstrate that chronic treatment with ethanol in rats results in a reduction of presynaptic cholinergic markers in various brain regions paralleled by an impairment in memory which can be ameliorated through pharmacological manipulation of cholinergic neurotransmission by physostigmine.

A severe deficiency of the basal forebrain cholinergic projection system both at the site of cholinergic somata in the basal forebrain and at the site of axon terminals in target areas, such as cortex and hippocampus, is in agreement with previous biochemical and morphological studies on the neurotoxic effects of chronic ethanol in rodent (2, 6, 8, 9, 29) and human (1, 4, 12, 31-33). Ethanol induced changes, however, are not restricted to cholinergic projection neurons of the basal forebrain. Cholinergic interneurons, such as those of the striatum, are affected to a similar extent as demonstrated in this and previous studies (2, 35). Furthermore, the same regime of ethanol treatment, as used in the present study, results in reductions of the content of other neurotransmitters such as noradrenaline, serotonin and dopamine throughout the brain (2). Thus, the deficiency of the cholinergic afferentation of the cortical mantle induced by chronic ethanol is embedded in a multisystem dysfunction. Although a quantitative relationship could be demonstrated between memory impairment in rat after chronic ethanol application, and damage of the cholinergic basal forebrain projection system (5), a causal link is difficult to establish.

Ameliorative effects of physostigmine, but not neostigmine, on the memory impairment induced by ethanol treatment support the hypothesis of an involvement of cholinergic neurons in the mediation of the ethanol-induced behavioural deficiency. This is in agreement with previously reported effects of physostigmine on behavioural impairments in rodent induced by ethanol application (8, 17, 18). Furthermore, physostigmine has been proven to be effective in the clinical management of the cognitive performance in amnesic patients including those with acute
alcoholic psychosis (13, 26). A further line of evidence in support of the relationship between cholinergic dysfunction and ethanol induced memory disturbance derives from the comparison of the nature of neuro-psychological deficiencies in various amnesic disorders and after pharmacological blockade of cholinergic activity. Postalcoholic Korsakoff's syndrome is primarily characterized by an acquisition or learning deficit and resembles in this respect the amnesia induced by the application of anticholinergic drugs to healthy subjects (24, 25). Under both conditions, aspects of long-term secondary memory are severely affected while those of short-term primary memory are almost spared. This clinical observation is matched by the finding in the behavioural paradigm used in the present study where memory impairment after chronic ethanol application was detectable for long but not for short acquisition-retention intervals (8).

Psychopharmacological studies, using both animal (38) and human (14) subjects, indicate that cholinergic antagonists impair, and agonists improve cognitive function. More clear-cut evidence for the implication of defined populations of central cholinergic neurons in memory processes derives from animal experiments using stereotaxic lesioning techniques (2, 36). Studies of the behavioural effects of damage to forebrain cholinergic nuclei have demonstrated impairments in cognitive function, especially in tasks with memory load (36). Such impairments are seen after lesions in either the medial septal/diagonal band complex or the basal nucleus (2, 20, 22), thus affecting the cholinergic afferentation of the hippocampus and the cerebral cortex either alone or in combination. A qualitatively similar impairment in long term aspects of spatial memory components, however, have been obtained in rat after placing excitotoxic lesions either to nuclei of the cholinergic basal forebrain projection system or to the striatum (Arendt, Sinden, Schugen, Marchbanks, Gray; in preparation). Chronic ethanol results in a reduction of pre-synaptic cholinergic markers in both the basal forebrain and the striatum as well as in an impaired performance on long term memory aspects in a behavioural task with a spatial memory load. While the present biochemical and psychopharmacological data add further support to the hypothesis of a cholinergic genesis of ethanol-induced memory impairment, they do not allow one to determine on the extent to which it can be attributed to a dysfunction of cholinergic projection neurons of the basal forebrain or to cholinergic interneurons of the striatum. The results, however, demonstrate that pharmacological manipulation of cholinergic activity can ameliorate behavioural sequelae of chronic brain damage in an animal model which involves neuronal death in the basal forebrain cholinergic projection system to a comparable extent (4-8) as
in amnesic/dementing disorders such as Korsakoff's syndrome or early Alzheimer's disease.

REFERENCES


