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European Journal of Pharmacology 313 (1996) 69–72

ejp

Short communication

Ethanol alone or with dexamethasone alters the kinetics of choline acetyltransferase

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Received 19 February 1996; revised 24 July 1996; accepted 26 July 1996

Abstract

Choline acetyltransferase activity was measured in rats treated with daily injections of ethanol (0.1 g/kg body wt) and or dexamethasone (1 mg/kg body wt) for 5 consecutive days. Ethanol produced a biphasic reduction of choline acetyltransferase activity in rat cerebral cortex, which at most time points was further decreased by simultaneous injection of dexamethasone. Kinetic studies of cortex choline acetyltransferase activity in rats that had received 5 daily injections of ethanol or ethanol and dexamethasone indicated that the observed reduction in enzyme activity was due to an apparent reduction in affinity (K_m) of the enzyme for acetyl coenzyme A with no significant change in the total amount of enzyme present (V_{max}). This finding has implications with respect to the use of choline acetyltransferase as a marker for cholinergic neurons, and for the understanding of the regulation of choline acetyltransferase activity in the brain.

Keywords: Choline acetyltransferase; Ethanol; Dexamethasone; Acetyl coenzyme A; Enzyme affinity; Stress

1. Introduction

Choline acetyltransferase (EC 2.3.1.6) catalyzes the synthesis of acetylcholine both in the peripheral and central nervous systems. Because of its specific functional role and specific localization, it has been used extensively as a marker for cholinergic neurons. Consequently, a reduction in choline acetyltransferase activity is often considered an indication of depletion of cholinergic neurons and cholinergic function in the brain. This view is reinforced by the fact that during development and in the adult rat there is a close correlation between the amount of choline acetyltransferase mRNA and the level of choline acetyltransferase activity both in normal and NGF-stimulated animals (Cavicchioli et al., 1991). Transcriptional activity of the choline acetyltransferase gene would thus seem to be the main mode of regulation of the expression of choline acetyltransferase activity. However, it is also known that certain post-transcriptional events, such as alternative splicing, phosphorylation and subcellular translocation, can affect choline acetyltransferase activity (Wu and Hersh, 1994). It is therefore important to determine whether an observed decrease of choline acetyltransferase activity is due to a reduction in gene expression, a change in enzyme affinity induced by a post-translational event, or the death of cholinergic neurons. Such information would be useful for formulating the necessary treatment for alcohol-induced choline acetyltransferase changes.

In this study we focused on the effect of ethanol and glucocorticoids on choline acetyltransferase activity. The interest in the interaction of these two substances arises from the fact that alcohol abuse in humans often occurs during periods of stress when glucocorticoid levels are presumably elevated. Previous reports have indicated that both glucocorticoids (Finkelstein et al., 1985; Bau and Vernadakis, 1982; Brown et al., 1988) and ethanol (Carmmichael and Israel, 1975; Brodie and Varnadakis, 1992) can reduce or increase choline acetyltransferase activity depending on the dose given, route of administration and brain region studied. We aimed to further characterize the

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PII S0014-2999(96)00601-2

nature of ethanol- and dexamethasone-induced changes of choline acetyltransferase activity and found that at relatively high chronic doses these drugs caused a decrease in choline acetyltransferase affinity (K_m) for its substrate acetyl coenzyme A (acetyl CoA) without significantly affecting the maximal enzyme velocity (V_{max}).

2. Materials and methods

Male Sprague-Dawley rats (8 weeks old) were housed 5 per cage on a 12 : 12 h light-dark cycle with water and food ad libitum. Injections of ethanol (10% v/v, 0.1 mg/kg body wt) and dexamethasone (1 mg/kg body wt) dissolved either in ethanol (10% v/v) or normal saline or sham injections, were given subcutaneously once a day between 09:30 and 10:30 h. Animals were killed 24 h after the last treatment, and the brains were dissected and kept at −20°C until used. Choline acetyltransferase activity was determined as described by Fonnum (1975). Briefly, thawed brain tissue was homogenized in ice-cold 50 mM sodium phosphate buffer (10% w/v, pH 7.4) containing 1 mM EDTA. Homogenate aliquots (10 μl) were added to 10 μl of the assay mixture consisting of 50 mM sodium phosphate (pH 7.4), 10 mM choline chloride, 300 mM sodium chloride, 10 mM EDTA, 0.1 mM eserine, 0.5 mM acetyl CoA and [^{3}H]acetyl CoA (0.04–0.08 μCi per tube). After 15 min at 37°C the reaction was terminated by adding 2.5 ml of 10 mM sodium phosphate buffer (pH 7.4) and 1 ml of butyronitrile containing 15 mg of tetraphenylboron. The solutions were thoroughly mixed and separated by centrifugation at 3000 × g for 4 min. An aliquot of the upper organic layer was transferred to a scintillation vial containing 5 ml of scintillation fluid and 2 ml of acetonitryl and the radioactivity was counted by a liquid scintillation counter at 45% efficiency. Choline acetyltransferase activity was expressed as nmol of [^{3}H]acetylcholine formed per minute per mg protein. Protein determination was carried out according to the method of Lowry et al. (1951) using bovine serum albumin (fraction V; Sigma Chemicals, St Louis, MO, USA) as a standard. Unless indicated, all values are given as means ± S.E.M. Statistical significance was estimated by two-tailed Student's t-test.

3. Results

Choline acetyltransferase activity was significantly reduced in the three brain regions studied, cortex, striatum and hippocampus, after the 5-day treatment with ethanol (0.1 g/kg body wt) or dexamethasone (1 mg/kg body wt) as compared to sham-injected animals (Fig. 1a). In the cortex and striatum, but not in the hippocampus, combined injections of ethanol plus dexamethasone caused a further significant reduction of choline acetyltransferase activity as compared to ethanol alone. Choline acetyltransferase activity in the cortex was reduced in a biphasic manner by daily injections of either ethanol or ethanol plus dexamethasone (Fig. 1b). The greatest reduction was observed at day 3; however, at this time dexamethasone atypically produced a partial, but not statistically significant, reversal of the ethanol-induced reduction of choline acetyltransferase activity. We chose to perform further studies of choline acetyltransferase activity after 5 daily injections as, at this time point, we observed a significant ethanol-induced or dexamethasone-induced reduction of choline acetyltransferase activity and a further significant reduction by ethanol plus dexamethasone.

Kinetic studies of choline acetyltransferase activity were performed by varying the concentration of acetyl CoA in the assay of brain cortex homogenate of rats treated for 5 days with either sham injection, ethanol or dexamethasone dissolved in ethanol. The K_m of choline acetyltransferase for acetyl CoA of control animals was 35.20 μM, which is similar to the previously reported value of 46.5 μM (Ryan and McClure, 1980). Table 1 shows that there was a significant decrease in affinity (increase in K_m) of choline acetyltransferase for acetyl CoA in brain cortex tissue from rats treated with ethanol and a further significant decrease in rats treated with ethanol and dexamethasone. In contrast, the maximal velocity of choline acetyltransferase

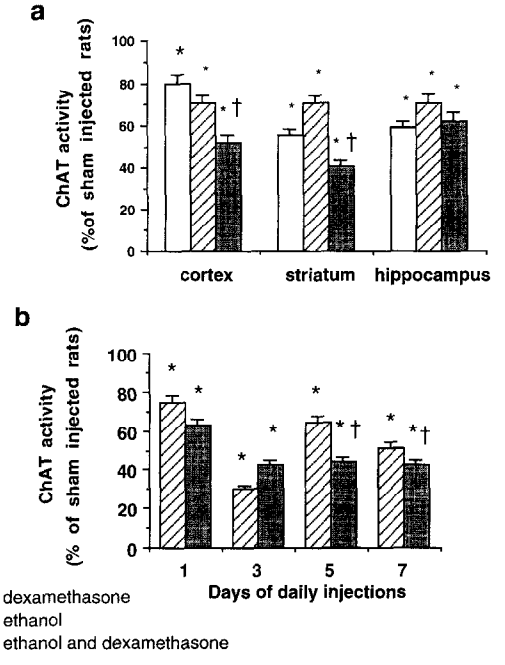

Fig. 1. Effect of ethanol and dexamethasone on choline acetyltransferase enzyme activity. Rats were injected subcutaneously with ethanol (0.1 g/kg body wt, hatched bars), dexamethasone (1 mg/kg body wt, open bars) or ethanol and dexamethasone for 5 consecutive days (a) or for the indicated number of days (b). Choline acetyltransferase activity was measured in homogenates of either the indicated tissues (a) or cortex (b) by the assay described in Section 2. Means ± S.E.M. of four separate experiments (for each point $n = 5$) are reported. * Significantly different from sham-injected rats ($P < 0.05$). † Significantly different from ethanol-injected rats ($P < 0.05$).

Table 1
Effect of ethanol and ethanol plus dexamethasone on the kinetic properties of rat cortex choline acetyltransferase enzyme

Drug treatments [a] (mg/kg body wt)	K_m [b] (μM)	V_{max} [b] (nmol · min^{-1} · mg protein^{-1})
Sham injection	35.20 ± 1.40	3.43 ± 0.50
Ethanol (100)	84.10 ± 5.18 [c]	2.77 ± 0.15
Ethanol (100) dexamethasone (1)	125.38 ± 17.4 [c,d]	4.07 ± 0.54

[a] Drugs were injected subcutaneously for 5 consecutive days.
[b] K_m and V_{max} values were obtained from double-reciprocal plots of initial velocity of choline acetyltransferase enzyme activity in presence of concentrations of acetyl CoA ranging from 5 to 50 μM. Choline acetyltransferase assay was performed as described in Section 2. Means ± S.E.M. of two experiments (for each group, $n = 4$) are reported.
[c] Significantly different from sham injection ($P < 0.05$).
[d] Significantly different from ethanol injection ($P < 0.05$).

enzyme (V_{max}) was not significantly different among these three groups.

4. Discussion

In all brain regions studied it was shown that choline acetyltransferase activity was sensitive to chronic injection of ethanol and of dexamethasone (Fig. 1a). In contrast to the finding in the cortex and striatum, in the hippocampus the combined effect on choline acetyltransferase activity of the two drugs was not significantly different from that of either drug alone, indicating that there might be different synergistic interactions between dexamethasone and ethanol in different brain regions. However, as the two drugs were injected as a mixture, ethanol may have affected the diffusion rate of dexamethasone and thus it is not possible to make firm conclusions about the potential additive or synergistic effect of the two drugs. In this work dexamethasone was used to mimic the typical hormonal response induced by environmental stress the rationale being that alcohol consumption by many individuals is increased during periods of stress. We did not use dexamethasone in order to mimic ethanol-induced cellular stress in the brain. Ethanol and dexamethasone were thus treated as two different drugs having a similar but complex effect on choline acetyltransferase enzyme.

The complexity of the effect of ethanol and dexamethasone on choline acetyltransferase activity is further exemplified by the observed biphasic nature of the reduction of choline acetyltransferase activity over the first days of injections. The time-dependent changes here reported for the cortex were also observed in the striatum but not in the hippocampus, again indicating a difference in this region with regard to susceptibility to ethanol and dexamethasone, possibly due to hippocampus-specific neuronal circuitry.

The kinetic studies of cortex choline acetyltransferase enzyme indicate that the observed reduction in choline acetyltransferase activity is due to a reduction in affinity (K_m) for acetyl CoA. In order to exclude the possibility that the observed reduction in choline acetyltransferase activity was caused by the presence of residual amounts of ethanol, dexamethasone or one of their metabolites still present in the tissues of the treated animals, ethanol (up to 200 μg/μl) or tissue homogenate (10 μl) from treated animals was added directly in the assay mixture of control animals. Neither method significantly reduced total choline acetyltransferase activity, indicating that the observed change of activity was due to a modification of the enzyme itself. We here report (Table 1) a change in choline acetyltransferase K_m for ethanol- and ethanol and dexamethasone-treated animals. We did not perform a similar kinetic study for animals injected with dexamethasone alone, but given that the V_{max} did not change in either ethanol- or ethanol and dexamethasone-treated animals, it can be assumed that the dexamethasone effect on choline acetyltransferase was also due to a change in K_m.

We do not wish to over-interpret our results as an explanation of the observed reduction in choline acetyltransferase affinity here described would entail a detailed investigation of transcriptional and post-translational events for this enzyme. Recent studies on the molecular mechanisms involved in the production of choline acetyltransferase have revealed that choline acetyltransferase is encoded in the rat, as in other species, by a single gene (Ibanez and Persson, 1991). Alternative splicing of the precursor RNA results in the production of several m-RNAs that differ in the 5′ untranslated region, but not in the coding region (Wu and Hersh, 1994). However, a cDNA with an alternative translation starting site has been isolated from human cells in culture (Oda et al., 1992), which could potentially give rise to two enzyme proteins with different specific activities. Although it is not known whether this phenomenon also occurs in the rat, the presence of isozymes with a different specific activity could represent an explanation of the observed ethanol- and ethanol plus dexamethasone-induced changes in affinity, assuming that the drugs differentially affected the expression of one of the isozymes. An alternative explanation for the observed changes in choline acetyltransferase affinity is a drug-induced change in post-translational processes. It has been shown that the kinetic properties of recombinant rat choline acetyltransferase can be altered by phosphorylation (Habert et al., 1992); however, the physiological role of phosphorylation of choline acetyltransferase has not been demonstrated, nor it is known whether ethanol or dexamethasone can affect the phosphorylation of choline acetyltransferase. It has also been reported that a choline acetyltransferase enzyme with different biological properties may exist in a membrane-bound form. Thus, although the relevant published data are controversial (Wu and Hersh, 1994), translocation of choline acetyltransferase to a different cellular compartment could also provide an explanation for the observed change in affinity.

In summary, we have observed that treatment with

ethanol and dexamethasone causes in rats a decrease in choline acetyltransferase activity which is apparently due to a reduction in affinity of the enzyme. The implications for alcohol abusers are that ethanol reduces choline acetyltransferase activity and that this effect is increased in states where there is an increase of circulating corticosteroids such as during environmental or psychological stress. However, the mechanism by which ethanol and dexamethasone, either directly or via other neurotransmitter systems, alter transcriptional or post-translational events that affect choline acetyltransferase activity remains to be elucidated. The finding that the change in activity is due to a change in affinity rather than a reduction of total enzyme concentration is of importance with respect to the general use of this enzyme activity as a marker for cholinergic neuron viability. It remains to be seen whether this effect on choline acetyltransferase can be reversed either by time or by specific drug treatments.

Acknowledgements

We would like to thank Mr. W. Longworth for assistance in the manuscript preparation and Mahidol University for financial support.

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