RAPID COMMUNICATION

NITRIC OXIDE IS NOT INCREASED IN ALCOHOLIC BRAIN

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Abstract — Nitric oxide (NO) metabolites nitrite and nitrate were measured in the cerebrospinal fluid in 12 alcohol-dependent subjects and in 16 healthy controls. The amounts of NO metabolites in alcoholics were not different from those in the controls. The results suggest that NO is not a major factor responsible for brain damage in these patients.

INTRODUCTION

It is now well established that nitric oxide (NO) is a mediator of neuronal damage in experimental 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism (Hantraye et al., 1996). It is also suggested that NO is involved in neurotoxic damage in stroke, Parkinson’s disease and Alzheimer’s disease (Snyder, 1996).

Neuronal NO synthases (nNOS), which catalyse the formation of NO, are abundant in several brain locations, some of which are involved in alcohol-induced brain damage, e.g. cerebellum, cortex and hippocampus (Bredt et al., 1990). Furthermore, NO appears to mediate glutamate neurotoxicity (Garthwaite, 1991), which may be involved in alcohol-induced brain damage (Lancaster, 1992). There is some experimental evidence suggesting that NO may be involved in alcohol drinking and preference regulation in experimental animals (Beaugé et al., 1994) and may also modulate other ingestive behaviours (Morley and Flood, 1991).

The objective of the present study was to investigate whether NO formation in the brains of alcohol-dependent subjects was different from that in non-alcoholics. We measured the amount of nitrite, a stable spontaneous oxidation product of NO in non-haemoglobin solutions, and nitrate, another conversion product of NO, in the cerebrospinal fluid (CSF) in a group of sober alcohol-dependent subjects and non-alcoholic controls.

METHODS

Twelve patients who fulfilled DSM-III-R criteria for alcohol dependence (American Psychiatric Association, 1987) were included. Three of them were smokers consuming 10–20 cigarettes per day. The mean duration of the last binge before the admission was 25 days, their estimated mean alcohol consumption during the binge was 222 g per day. The withdrawal symptoms were monitored by recording the Clinical Institute Withdrawal Assessment of Alcohol (CIWA-A) scale (Shaw et al., 1981). CSF was collected at 10–16 days, after the alcohol withdrawal treatment with oxazepam was completed and CIWA-A scores had reached zero.

Sixteen healthy volunteers were used as controls. The control subjects reported drinking only small amounts of alcohol. The CAGE scores (Mayfield et al., 1974) for all subjects were zero. One of the control subjects smoked 10 cigarettes per day, and another used snuff tobacco. The study...
Table 1. Nitrite and nitrate concentrations in CSF of alcohol-dependent patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Nitrite (µmol/l)</th>
<th>Nitrate (µmol/l)</th>
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<tbody>
<tr>
<td>Alcohol-dependent subjects $\text{(n = 12)}$</td>
<td>$0.26 \pm 0.03$</td>
<td>$7.28 \pm 0.40$</td>
</tr>
<tr>
<td>Control subjects $\text{(n = 16)}$</td>
<td>$0.19 \pm 0.15$</td>
<td>$7.54 \pm 0.66$</td>
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</table>

Values are given as means $\pm$ SEM. Differences between patients and controls did not attain statistical significance ($P > 0.05$).

The protocol was approved by the Karolinska Hospital Ethics Committee. The trial participants were aware of the experimental nature of the study and informed consent was obtained from each.

CSF was collected 4 h after a light breakfast. The smokers had ceased smoking at least 4 h before CSF sampling. No artificial bleeding occurred during the lumbar puncture. CSF was analysed for nitrite and nitrate using gas chromatography-mass spectrometry according to methods described elsewhere (Wennmalm et al., 1994).

The results are presented as means $\pm$ SEM. Statistical differences between the subject groups were evaluated using Student’s $t$-test for unpaired observations. Correlations between study variables were calculated with the aid of Pearson’s correlation coefficients.

RESULTS

The values of nitrite and nitrate of alcohol-dependent patients and healthy non-alcoholic control subjects are shown in Table 1. The nitrite values in the alcoholic subjects were slightly higher when compared with those of the control subjects, but the difference did not reach statistical significance ($P > 0.05$). The values of CSF nitrate in alcohol dependent subjects were also not different from those of the controls. There was no correlation between the amount of alcohol consumed before admission or the interval before CSF sampling and CSF NO metabolites.

DISCUSSION

Nitrite, a stable breakdown product of NO, is considered to reflect the formation of NO in aqueous solutions, such as CSF (Moncada and Higgs, 1993; Wennmalm et al., 1994). The major metabolic transformation of NO into nitrate is by means of haemoglobin. In healthy sober alcohol-dependent subjects, haemoglobin is not a constituent of CSF. However, small amounts of nitrate are formed in the CNS. Since the brain and spinal cord are bathed in CSF, both nitrite and nitrate reflect the NO production in the CNS. However, by measuring NO metabolites in CSF, NO production in different parts of the CNS and the contribution of NO from sources outside the CNS cannot be evaluated.

The alcohol-dependent subjects were investigated after a short period of abstinence. The duration of abstinence appeared not to influence NO formation. Moreover, the amount of alcohol consumed before admission was not correlated to the measures of NO formation. The effect of oxazepam on NO synthesis in the CNS is still unknown. The patients had discontinued the treatment with oxazepam 10–16 days before the CSF sampling. It is therefore unlikely that the withdrawal treatment had any effect on the amounts of NO metabolites measured in the CSF in our study.

It has recently been shown that inhibition of nNOS by a specific inhibitor, 7-nitroindazole, prevents MPTP-induced parkinsonism in experimental animals (Hantraye et al., 1996). These findings indicate an important role for NO in mechanisms capable of causing brain dysfunction and damage. In our study, NO formation in the CNS in alcohol-dependent subjects appeared not to be different from that in controls. Therefore, the results of our study suggest that NO-mediated mechanisms are not operating in detoxified alcohol-dependent subjects. Moreover, if NO is involved in the mechanisms responsible for motivation to drink (Beaugé et al., 1994), they are not active in sober alcoholics. Further studies are needed to investigate whether NO formation in the brain is influenced by acute ingestion of alcohol as well as acute alcohol withdrawal.

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REFERENCES

American Psychiatric Association (1987) Diagnostic


