DIFFERENT EFFECTS OF SMOKING OR USE OF SMOKELESS TOBACCO ON PLATELET MAO-B ACTIVITY IN TYPE 1 ALCOHOL-DEPENDENT SUBJECTS

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Abstract — Background: Low platelet monoamine oxidase (MAO)-B activity has been proposed as a marker for alcohol-dependence. Findings are, however, contradictory and the influence of confounding factors have been thoroughly investigated. Thus, it is now well established that cigarette smoking reduces platelet MAO-activity. However, not much is known about the influence of smokeless tobacco, i.e. snuff or chewing tobacco, on platelet MAO-B activity. The aim of the present study was to compare platelet MAO-B activity in type 1 alcohol-dependent subjects with concomitant use of smokeless tobacco (i.e. snuff users), use of smoking tobacco (i.e. cigarette smokers), and in those without any tobacco use. Methods: Platelet MAO-B activity was examined in three groups of alcohol-dependent subjects: snuff users (n = 14), cigarette smokers (n = 33), and non-tobacco users (n = 5). In the alcohol-dependent subjects concomitant smokeless tobacco smokers, but not snuff users, were found to have significantly lower platelet MAO-B activity as compared to non-tobacco users (platelet MAO-B activity 4.0 ± 1.5, 5.1 ± 1.5 and 5.0 ± 1.9 µkat/kg protein, respectively). Conclusions: The findings in the present study suggests that in the alcohol-dependent subjects the concomitant use of smokeless tobacco, i.e. snuffing, does not have an inhibitory effect on platelet MAO-B activity. This may have implications for future research. Thus, alcohol-dependent subjects with concomitant tobacco use should be grouped separately according to the form of the tobacco used, i.e. smoking or smokeless tobacco.

INTRODUCTION
Platelet monoamine oxidase (MAO)-B activity has been investigated in numerous studies of alcohol-dependent subjects. Thus, in several studies platelet MAO-B activity has been found to be lower than that in controls and low platelet MAO-B activity has therefore been proposed to be a trait marker for alcohol-dependence, especially of type 2 alcoholism (Cloninger et al., 1981), which is associated with personality traits such as impulsiveness, sensation-seeking behaviour and monotony avoidance (Wiberg et al., 1977; Major and Murphy, 1978; Sullivan et al., 1978, 1990; von Knorring et al., 1984; Faraj et al., 1987; Pandey et al., 1988; Devor et al., 1993; Rommelspacher et al., 1994; Hallman et al., 1996; von Knorring and Oreland, 1996; Oreland, 2004). In a recent study by Snell et al. (2002), it has furthermore been found that low platelet MAO-B activity in subjects with a history of life-time alcohol dependence can be attributed to low platelet MAO-B concentration. However, in other studies, of which some included type 2 alcoholics, platelet MAO-B activity was not different from that in controls (Tabakoff et al., 1988; Parsian et al., 1995; Anthenelli et al., 1998; Farrer et al., 1998; Soyka et al., 2000; Whitfield et al., 2000). Nevertheless, in the recent review by Oreland (2004) associations between low platelet MAO-B activity and personality traits, such as sensation seeking and impulsiveness, and type 2 alcoholism are convincingly demonstrated. Strong support for the association of low platelet MAO-B activity and excessive alcohol consumption and type 2-like alcohol features has also been found in non-human primates (Fahlke et al., 2002). In studies of alcohol-dependent subjects, in which a family history of alcoholism has been taken into account, most studies have found lower platelet MAO-B activity in family history positive (FHP) in comparison to family history negative (FHN) alcohol-dependent subjects (Major and Murphy, 1978; Sullivan et al., 1979; Alexopoulos et al., 1980; Rommelspacher et al., 1994). However, Soyka et al. (2000) reported no difference in platelet MAO-B activity between FHP and FHN alcohol-dependent subjects and Snell et al. (2002) found no influence of family history of alcoholism on platelet MAO-B activity when using multiple regression analysis.

Summarizing the findings of the studies of platelet MAO-B activity in alcohol-dependent subjects the results have thus been contradictory. It is therefore not surprising that in recent years the influence of potential confounding factors on platelet MAO-B activity in alcohol-dependent subjects has been intensively discussed. Among such potential confounding factors gender, temporal change in platelet MAO-B activity after end of alcohol intake and smoking status, have been thoroughly investigated.

With respect to gender it has been found in three studies, consisting of a large number of subjects, that females have higher platelet MAO-B activity than males (Anthenelli et al., 1998; Whitfield et al., 2000; Snell et al., 2002). In the study of Snell et al. (2002), a possible explanation for the gender difference, as to why females were found to have higher platelet MAO-B concentrations than males, is also offered.

Considering the temporal pattern of platelet MAO-B activity after end of alcohol intake, it has been shown that there may be transient fluctuations in platelet MAO-B activity several months after end of alcohol intake (Wiberg et al., 1977; Major et al., 1981; Alexopoulos et al., 1981; Berggren et al., 2000). To exclude this potential confounding factor of transient changes in platelet MAO-B activity during the first months after end of alcohol intake, we have earlier investigated platelet MAO-B activity in long-term abstinent type 1 alcohol-dependent subject. These subjects, who had an abstinence period of 7±6 years (minimum 1 year), i.e. were
alcohol-dependent subjects in sustained full remission, did not differ from controls in platelet MAO-B activity (Berggren et al., 2002a). It may therefore be concluded that, when the potential confounding factor of transient changes in platelet MAO-B activity after end of alcohol intake has been eliminated, there is no difference in platelet MAO-B activity between alcohol-dependent subjects, at least those who could be characterized as type 1 alcoholics, and controls (Berggren et al., 2002a).

Regarding smoking status it has been reported in three recent studies, consisting of a large number of subjects, that current smoking reduces platelet MAO-B activity (Anthenelli et al., 1998; Whitfield et al., 2000; Snell et al., 2002). It has thus been suggested that low platelet MAO-B activity is a state marker for cigarette smoking rather than a trait marker for alcoholism or its subgroups (Anthenelli et al., 1998; Whitfield et al., 2000). This view is, however, not supported by the findings in the study of Snell et al. (2002).

In that study subjects with a life-time history of alcohol dependence had lower platelet MAO-B activity, independent of smoking status. Furthermore, in studies that have taken the potential confounding factor of smoking status into account, when comparing platelet MAO-B activity between FHP and FHN alcohol-dependent subjects, there was no difference between these groups (Whitfield et al., 2000; Berggren et al., 2002b; Snell et al., 2002). Concerning the inhibitory effect of smoking on platelet MAO-B activity, it has been suggested that this effect is probably not due to an inhibitory effect of nicotine but rather to some other substance of the several thousand compounds in cigarette smoke (Yu and Boulton, 1987; Anthenelli et al., 1998; Orelend et al., 1999; Fowler et al., 2000; Castagnoli et al., 2001; Snell et al., 2002; Oleland, 2004).

Consequently, when studying platelet MAO-B activity in alcohol-dependent subjects the influence of confounding factors must be taken into consideration. Concerning the influence of tobacco use on platelet MAO-B activity it is now, as mentioned above, well established that the use of smoking tobacco, i.e. cigarette smoking, inhibits platelet MAO-B activity. However, not much is known about the influence of smokeless tobacco, i.e. snuff or chewing tobacco, on platelet MAO-B activity. The aim of the present study was therefore to investigate platelet MAO-B activity in three groups of male alcohol-dependent subjects: snuff users, cigarette smokers, and non-tobacco using individuals.

METHODS

Subjects

Male subjects aged 20 to 65 years were recruited by advertisement in a daily newspaper. Inclusion criteria were social stability, i.e. employment or living on a pension and with a permanent place of residence. They also had to be without any physical or psychiatric disorder not associated with alcohol intake. Diagnoses of abuse or dependence of substances other than alcohol and nicotine were not permitted. Their weekly alcohol consumption should have exceeded 300 grams pure alcohol during the last 2 weeks before the investigation.

Study design

The subjects, who were all found to be without a history of treatment as in-patients for alcohol-related problems, were examined physically and psychiatrically by a psychiatrist at an alcoholism and drug treatment unit of the University Hospital through a semi-structured interview. They were assessed for alcohol-abuse/dependence according to the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) criteria of the American Psychiatric Association (1994). The subjects were also assessed for type 1 alcoholism using the criteria of von Knorring et al. (1985): subjective alcohol problems should have started after the age of 25 and the first treatment contact should have been established after the age of 30. Or, if the alcohol problems had started earlier, there should be no signs of social complications such as violence while intoxicated, absence from work due to alcohol, loss of job, legal difficulties (e.g. arrest for intoxication or traffic accidents while intoxicated), arguments or difficulties with family or friends because of excessive alcohol abuse.

Alcohol consumption and tobacco use

During the last 2 weeks before blood sampling the daily alcohol consumption was registered by alco-cards (Ballin et al., 1994), Time Line Follow Back (Sobell et al., 1980) or daily telephone calls (Eriksson et al., 2001). The subjects were also requested to estimate for what time period (in years) they had had an excessive level of alcohol consumption. Thereafter the subjects’ age at onset of excessive alcohol consumption was calculated and recorded. Past-year tobacco status was registered as number of cigarettes per day and snuffing (i.e. tucking snuff under the lip) as amount (grams) of snuff used daily.

Biochemical tests

Blood samples were collected at the investigation day for the determination of platelet MAO-B activity and liver function parameters (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT); upper laboratory limit for all liver enzymes: 0.8 µkat/L). Presence of illicit drugs and benzodiazepines in urine samples was also assessed, using suitable laboratory screening procedures.

The subjects were currently drinking alcohol but were instructed to end their alcohol intake the day before the investigation. The subjects who were tobacco users were instructed not to use tobacco after midnight before the night before the determination of platelet MAO-B activity.

Biochemical analyses

Platelets were isolated from EDTA-blood (Svennerholm et al., 1982) and the cell homogenate, used as enzyme source in the assay of MAO-B, was prepared by sonication in ice-chilled water for 60 s. The protein content of the homogenate was determined, there is no difference in platelet MAO-B activity after end of alcohol intake has been eliminated, is a state marker for cigarette smoking rather than a trait marker for alcoholism or its subgroups (Anthenelli et al., 1998; Whitfield et al., 2000).
The intra-assay variation was 4 to 6%, calculated on assayed duplicates of different samples. For determination of the inter-assay precision, a pool of platelets was aliquoted and stored at −80°C. Fresh aliquots were then assayed on seven different occasions during a 5-month period. The inter-assay variation of the pooled platelet samples was 10%.

Statistical analyses
Analysis of variance (ANOVA) was used to detect an overall effect and Fisher’s Protected Least Significant Difference (PLSD) was used for post-hoc comparisons between the groups. When analysing correlation between data univariate Pearson’s product-moment correlation was used. In all tests, two-tailed levels of significance were used. The data are presented as mean ± standard deviation (SD).

This study was approved by the Ethics Committee of the Göteborg University, Sweden and was in compliance with the Helsinki Declaration of 1975. Informed and written consent was obtained from all subjects. None of the subjects were paid for their participation in the study.

RESULTS
All subjects were found to have alcohol-dependence according to DSM-IV (American Psychiatric Association, 1994). All subjects also exclusively met the criteria according to vom Knorring et al. (1985) for type 1 alcoholism. Thus, all of them self-reported that their subjective alcohol problems had started after the age of 25 and in those who reported treatment contact this had been established after the age of 30. In fact, the majority of the subjects (about 80%) had no established treatment contact at the time for the investigation. The mean ± SD age of the 93 subjects was 50 ± 6 years. Their daily alcohol consumption the last 2 weeks before blood sampling was 100 ± 44 grams pure alcohol. The values for liver function parameters AST, ALT and GGT were 0.5 ± 0.3, 0.7 ± 0.4 and 1.4 ± 1.5 μkat/L, respectively. None was found to be positive in the urine screening tests for narcotic drugs or bensodiazepines. Fourteen subjects (15%) were snuff users, 33 (35%) cigarette smokers and 46 (50%) non-tobacco users. Background characteristics for the three groups are given in Table 1.

All subjects self-reported (alco-cards, Time Line Follow Back or daily telephone calls) that they had ended their alcohol intake the day before the investigation of the platelet MAO-B activity. This was also confirmed by negative breath analyser tests. None of the subjects showed any signs of alcohol withdrawal syndrome.

Platelet MAO-B activity in snuff users was not different from that in non-tobacco using alcohol-dependent subjects, 5.1 ± 1.5 and 5.0 ± 1.9 μkat/kg protein, respectively (Fig. 1). Platelet MAO-B activity in smokers was 4.0 ± 1.5 and this value was significantly lower than that in snuff users and non-tobacco using alcohol-dependent subjects (P = 0.05 and P < 0.01, respectively).

In the snuff users there was no correlation between the amount of snuff used per day (28 ± 12 grams) and platelet MAO-B activity. Neither was there any correlation in the quantity of snuff used per day (28 ± 12 grams) and platelet MAO-B activity. Neither was there any correlation in the.

smokers between the number of cigarettes used per day (21 ± 13) and platelet MAO-B activity.

DISCUSSION
It is now well established that the use of smoking tobacco, i.e. cigarette smoking, inhibits platelet MAO-B activity, probably by the presence of inhibitory compounds in cigarette smoke (Anthenelli et al., 1998; Whitfield et al., 2000; Snell et al., 2002; Oreland, 2004). However, not much is known about the influence of smokeless tobacco, i.e. snuff or chewing tobacco, on platelet MAO-B activity. Therefore, we have in the present study of 93 male type 1 alcohol-dependent subjects examined platelet MAO-B activity in snuff users and compared it to that of cigarette smokers and non-tobacco users. Only male subjects were included in this study in order to avoid the confounding effect of gender on platelet MAO-B activity, i.e. females have higher platelet MAO-B than males (Anthenelli et al., 1998; Whitfield et al., 2000; Snell et al., 2002).

### Table 1. Background characteristics for smoking (N = 33), snuffing (N = 14) and tobacco non-using (N = 46) type 1 alcohol-dependent individuals. Data are presented as mean ± SD (range)

<table>
<thead>
<tr>
<th></th>
<th>Smokers</th>
<th>Snuffers</th>
<th>Tobacco non-users</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) at investigation</td>
<td>48 ± 6</td>
<td>50 ± 7</td>
<td>51 ± 7</td>
</tr>
<tr>
<td>Age at onset of excessive alcohol consumption</td>
<td>38 ± 9</td>
<td>39 ± 12</td>
<td>41 ± 9</td>
</tr>
<tr>
<td>Number of years of excessive alcohol consumption</td>
<td>10 ± 9</td>
<td>10 ± 9</td>
<td>9 ± 7</td>
</tr>
<tr>
<td>Alcohol consumption (grams pure alcohol per day) 2 weeks before the investigation</td>
<td>99 ± 4</td>
<td>114 ± 6</td>
<td>96 ± 4</td>
</tr>
<tr>
<td>Number of cigarettes used per day</td>
<td>21 ± 13</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Amount of snuff (grams) used per day</td>
<td>—</td>
<td>29 ± 12</td>
<td>—</td>
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</tbody>
</table>

Fig. 1. Platelet monoamine oxidase (MAO)-B activity in 93 type 1 alcohol-dependent male individuals. Shown are levels in non-tobacco users (N = 46), snuff users (N = 14) and in cigarette smokers (N = 33). Data are presented as mean ± SD. *: P = 0.05, **: P < 0.01 (Fisher’s Protected least significant difference [PLSD]).
In the present study, all subjects were currently drinking alcohol but were instructed to end their alcohol intake the day before the investigation. This was confirmed by self-reports (alco-cards, Time Line Follow Back or daily telephone calls). The subjects who were tobacco users were also instructed to not use tobacco after midnight before the investigation. Thus, the time-period that has elapsed between end of alcohol intake and tobacco use and the determination of platelet MAO-B activity is similar (less than 1 day) in all subjects. This is of importance since there is a well-documented increase in platelet MAO-B activity after end of alcohol intake (Major et al., 1981; Alexopoulos et al., 1987; Wiberg et al., 1977; Berggren et al., 2000) and if there is a difference in this time-period between groups that are compared for platelet MAO-B this may be a confounding factor.

The main finding in this study was that snuff users had platelet MAO-B activity not different from the non-tobacco using subjects. The current smokers were, on the other hand, found to have lower platelet MAO-B activity than both snuff users and non-tobacco using subjects. The reduction in platelet MAO-B activity by current smoking was about 20% in the present study, which is within the range of 14 to 53% that has been reported in other studies (see Snell et al., 2002). We found, however, no correlation between the number of cigarettes used per day and platelet MAO-B activity. This finding is at variance with that of Whitfield et al. (2000) and Snell et al. (2002) who did find such an association between the number of cigarettes used per day and platelet MAO-B activity, i.e. the larger number of cigarettes used per day the lower platelet MAO-B activity. It is, however, in agreement with the findings of Fowler et al. (2000) who reported no correlation between the number of cigarettes used per day and brain MAO-B activity. The findings in the present study could thus support the notion put forward by Fowler et al. (2000), suggesting that it is the smoking technique, rather than the number of cigarettes used per day that regulates the inhaled dose of nicotine and other substances during cigarette smoking.

A limitation for the finding in the present study of no difference in platelet MAO-B activity between the snuff users and non-tobacco using subjects may be the relatively small number of snuff users (N = 14) investigated. However, Alm et al. (1994) also found that non-alcohol-dependent snuff users (N = 10) had platelet MAO-B activity that was not different from that in controls. Thus, taken together the findings in the present study and that of Alm et al. (1994) suggest that the use of smokeless tobacco, i.e. snuff use, does not have an inhibitory effect on platelet MAO-B activity. This is probably explained by that nicotine itself does not have an inhibitory effect on platelet MAO-B activity (Oreland, 2004).

Another limitation of the study is that no other determinants of smoking status such as CO measurements and/or saliva cotinine measurements were used. This would have given a better estimate of smoke-exposure and perhaps better correlations with MAO-B activity.

The present finding may have implications for future research. Thus, when studying platelet MAO-B activity in alcohol-dependent subjects, those with concomitant tobacco use should be grouped separately according to the type of the tobacco used, i.e. smoking or smokeless tobacco.

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