CHAPTER 10

General discussion

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Alcohol is one of the most widely used psychoactive substances in Western society. This makes it important to study effects of this compound, and its interactions with other medications or drugs. Alcohol has complex and variable pharmacokinetics, and some of the effects of alcohol are preferably studied at stable serum levels. The results of studies investigating alcohol clearance or the effects of alcohol on the central nervous system (CNS) or drug-alcohol interactions are often interpreted more easily when alcohol levels are kept within certain limits. Especially, because both its wide inter- and intra-individual variability are reduced when alcohol is 'clamped' to a pre-specified level. Methods to obtain stable serum concentrations are scarce, but efforts have been made before (Hartmann *et al.*, 1988; O'Connor *et al.*, 1998). However, these methods have not gained wide application. This might be related to the perceived complexity of the procedures, which seem to require specific expertise and frequent alcohol concentration measurements for adaptations of the alcohol infusion. The early drug development process would benefit from an accurate, user-friendly method, with low variability, which is able to maintain constant alcohol levels for prolonged periods of time. Such a method would greatly facilitate any (drug) research in which alcohol is involved. In this thesis the development and application of a new relatively straightforward alcohol clamping procedure is presented.

Reliable studies of the CNS-effects of alcohol do not only benefit from stable alcohol conditions, but also from sensitive tests that are able to reliably detect the acute effects of alcohol. Chapter 2 of this thesis contains a systematic review, which attempted to determine the sensitivity to alcohol for a large number of CNS-tests that are described in the literature. The results show that many different tests or biomarkers are currently used to study the various CNS-effects of alcohol, and that such studies would greatly benefit from a certain degree of standardization. Attention tasks, visuo-motor control tests and scales of subjective effects were identified as the most sensitive functional biomarkers for the acute CNS-effects of alcohol. The results of this review are helpful in selecting rational tests for studies investigating the acute CNS-effects of alcohol or for future alcohol-interaction studies.

In chapter 3 we introduced the alcohol clamp. Based on the results of O'Connor's 'Indiana alcohol clamp' (O'Connor *et al.*, 1998) we developed a new method to maintain stable alcohol serum levels for prolonged periods of time. A large data-set of population pharmacokinetics was used to reduce the amount of sampling moments that O'Connor's method requires throughout the clamping period. Besides, we introduced an intravenous loading dose instead of the oral dose originally described by O'Connor. These adaptations were incorporated into a new spreadsheet-based paradigm, to enhance its user-friendliness. The new clamping method was compared to a method described by Hartmann (Hartmann *et al.*, 1988), which is based on individual alcohol pharmacokinetics to predict individual infusion rates to achieve a desired target level on a subsequent study occasion. We showed that the novel alcohol clamping paradigm was more accurate and user-friendly, with low variability and the ability to maintain constant alcohol levels for hours. The paradigm provided an opportunity to perform intensive pharmacodynamic or functional assessments during the execution of the clamp, which was considered to be useful for future studies of alcohol.

The modified alcohol clamping procedure described in Chapter 3 is based on fewer actual alcohol samples than the Indiana alcohol clamp procedure (O'Connor *et al.*, 1998). This may reduce the variability of alcohol levels at a steady-state, but the O'Connor method does not leave any room for concomitant pharmacodynamic testing, due to its frequent sampling moments. The adapted alcohol clamp as described in this thesis was partly developed to overcome this problem. In chapter 4 we showed that an intensive battery of CNS-tests could be integrated in the course of the clamp without interfering with the sampling activities or with the stability of the clamp. Furthermore, we showed that attention, subjective effects and (visuo-) motor control tests were mainly affected by alcohol. Also, we found that some effects closely followed the relatively stable alcohol concentrations, whereas others fluctuated during the plateau-phase, which could be an

indication for time-related drug-effect changes such as acute tolerance. Based on chapter 3 and 4 we conclude that our method provides careful control over BrAC-levels and allows frequent repetition of different CNS-measurements. These features make this technique eminently suitable to study the complex pharmacodynamic effects of acute alcohol administration.

During the alcohol clamp, the levels of ethanol that are infused in an attempt to maintain stable breath- and blood-levels provide detailed information about the individual disposition of the compound. Consequently, differences in the sensitivity to the effects of alcohol can be readily traced to pharmacokinetic and pharmacodynamic sources of variability. Japanese subjects are often more susceptible to the effects of alcohol, which is at least partly related to ethnic differences in alcohol metabolism but may also have pharmacodynamic causes. In chapter 5 we explored the pharmacokinetic and pharmacodynamic differences between a group of Caucasians and a group of Japanese healthy male volunteers at two different clamp levels at two different occasions. We found that significantly lower amounts of alcohol were needed for the Japanese group to maintain similar stable concentrations than for the Caucasians. However, these differences disappeared when values were corrected for lean body mass. Despite similar alcohol levels, several pharmacodynamic differences between the groups were observed, primarily on body sway and on the visual analogue scale (VAS) for subjective alcohol effects, and mainly at the highest dose level. We concluded that the alcohol clamp is a useful method to compare differences in alcohol metabolism between groups and that some CNS-effects of alcohol differed clearly between Japanese and Caucasians, but others did not, even though alcohol levels were stable and similar between the two groups.

The selection of CNS-tests within the CNS-battery we introduced in chapter 4 and 5 was based on a thorough literature review (chapter 2). Together, these tests cover a broad range of neuropsychological functioning. However, the results are only indirect read-outs of the acute effects of alcohol on the CNS. Ideally, a generally applicable methodology for repeated measurements of direct drug effects on the entire CNS, without task-related interactions and

a priori models, would constitute a major improvement in CNS drug development. Resting-state functional magnetic resonance imaging (RS-fMRI) could satisfy many of these requirements, and was hence hypothesized to be a promising technique for pharmacological research. The validity and sensitivity of this new method as an instrument in future CNS-drug development programs was explored in a proof-of-concept study, during which we used the alcohol clamp at a low level of 0.6 g/L, to calibrate the new technique under steady-state conditions (chapter 6). Besides alcohol, a morphine infusion was used on a different occasion to explore the specificity of the effects of CNS-depressants on RS-fMRI. Our results revealed dissociable changes in both pharmacodynamics and functional connectivity resulting from alcohol and morphine. *Post hoc* analysis of regions of interest revealed adaptive network interactions in relation to pharmacokinetic and pharmacodynamic curves for both alcohol and morphine. This study suggests that resting-state functional brain connectivity could play a role as a drug-class specific and –sensitive method in CNS drug research.

In the previous chapter, alcohol was used as a positive control for a new method to test CNS-active drugs, because it has a wide range of CNS-effects within clearly defined socially and clinically acceptable blood levels. Alcohol also has a limited number of established therapeutic effects, for instance on essential tremor and methanol intoxication, which makes it a useful positive control for novel therapies in these conditions. In chapter 7 and 8 we investigated the effects of a GABAa α2,3 subtype selective partial agonist (TPA023) and a histamine-3 inverse agonist (MK-0249) on essential tremor symptoms. In both studies we added an alcohol arm to the study design as a positive control. The alcohol clamp was used to obtain steady state breath alcohol levels. Although some CNS-effects were observed for both new compounds, no significant tremor reducing effects were reported. In contrast, the stable alcohol levels did result in significant reductions in tremor symptoms, and the alcohol clamp successfully fulfilled its role as a positive control in both studies. The alcohol clamp procedure seems a valid method to use in future studies investigating the effects of new therapeutic options in ET

research. Additionally, the alcohol clamp can be used during the validation process of new methods, which quantify essential tremor or assess tremor severity and its responsiveness to therapeutic interventions.

In this thesis a novel alcohol clamping method was introduced, which was developed to maintain stable serum levels of alcohol for prolonged periods of time by continuous intravenous administration, during which a BrAC input and feedback system is used, based on population pharmacokinetics. The procedure provides an environment in which the acute (CNS-)effects of stable alcohol levels can accurately be investigated, including the metabolic disposition of ethanol, the effects on a wide range of CNS-functions, and the changes in sensitivity across time. One drawback of the clamping paradigm is that alcohol levels do not show enough variation during the steady state to explore PK/PD relationships. However, prolongation of the clamp-run phase or attainment of multiple consecutive steady-state levels will provide the opportunity for future studies to investigate such relations in larger detail.

Our alcohol clamp might serve as an interesting tool in the early drug development process. We showed that alcohol clamp could be used a benchmark in proof-of-concept studies (chapter 6) and as a robust positive control during ET-studies (chapter 7 and 8). The alcohol clamp may also play a future role in the validation process of new tremor registration devices, which assist in the search for new pharmacological treatment strategies for tremor disorders. The clear legal limits of acceptable alcohol levels and its broad spectrum of CNS-effects also creates a useful framework for the clamp, to serve as a functional benchmark for the effects of new drugs, to get a feel for the safety and acceptability of inevitable CNS-effects in work and traffic and other domains of daily life.

For safety reasons, drug-alcohol interaction studies are incorporated into the course of the development program of most new CNS-drugs. Both pharmacokinetic and pharmacodynamic interactions should be explored to generate recommendations and/or restrictions for its future users. We believe that the alcohol clamp might be a helpful tool for drug-alcohol interaction studies, since steady state alcohol level will allow the measurement of multiple pharmacodynamic endpoints with reduced variability. The methods described in this thesis have already been implemented in successful drug-alcohol interaction studies (chapter 9). This thesis has examined several other examples where the alcohol clamp has been a useful research instrument to the advancement of alcohol research.

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