Pharmacoresistance in epilepsy : modelling and prediction of disease progression

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Chapter I

Scope and outline of the thesis

1.1 The problem of pharmacoresistance

Despite the availability of a wide range of antiepileptic drugs, in about one third of people with epilepsy seizures cannot be adequately controlled. Some never become seizure free after initiation of pharmacotherapy, while others may initially respond favourably, but eventually also become refractory to pharmacotherapy. Two major hypotheses have been proposed to explain the phenomenon of pharmacoresistance. The first suggests that alterations in transport across the blood-brain barrier restrict uptake of antiepileptic drugs in the brain, resulting in inadequate concentrations at the putative site of action. The second suggests that alterations occur in drug targets that reduce or eliminate the effectiveness of administered drugs. These two hypotheses are not mutually exclusive and both mechanisms may contribute to the development of pharmacoresistance. In studies in humans and in animal models of epilepsy numerous changes in transporters, ion channels, neurotransmitter systems, synaptic receptors and neuronal network properties have been uncovered which are consistent with either of the two hypotheses. Nevertheless, the exact mechanism causing the development of this pharmacoresistance has not yet been conclusively shown. It is possible that more than one mechanism contributes (in different degrees) to the development of pharmacoresistance.

As treatment of epilepsy requires regular administration of antiepileptic drugs, it is also possible that development of pharmacoresistance is due to chronic exposure to the drug. It can be argued, however, that mechanisms underlying the generation of epilepsy play an important, if not decisive, role in the development of pharmacoresistance. Firstly, in many patients seizures can not be controlled even at first diagnosis, suggesting that the mechanisms underlying pharmacoresistance were already in place before pharmacotherapy was started. Secondly, types of epilepsy associated with pharmacoresistance are often progressive, suggesting that the increasing severity of the disease is an important factor. A major obstacle in solving this problem is that it is not known at what stage of disease progression the changes in either drug targets or blood-brain barrier transport, or both, occur with sufficient impetus to result in pharmacoresistance. It is intriguing to challenge whether pharmacoresistance can be prevented by inhibiting progression of the disease. Obviously, a better understanding of the relationship between epileptogenesis and the development of pharmacoresistance will help to treat epilepsy by preventing the development of pharmacoresistance against antiepileptic drugs.

The investigations in this thesis focussed on the relationship between the time course

of epileptogenesis, associated changes in GABAergic inhibition and the development of pharmacoresistance. **It was hypothesised that the alterations in drug targets, leading to pharmacoresistance, originate shortly after the start of epileptogenesis, preceding or starting with the first epileptic insult.** To study cause, origin and time frame of pharmacoresistance development, *in vivo* studies were performed using animal models. This has the major advantage that induction of epilepsy, and therefore the start of the epileptogenic process, is controllable. Moreover, in principle, the full course of epileptogenesis can be followed, using specific biomarkers that reflect the process of epileptogenesis. The use of quantitative biomarkers, such as positron emission tomography (PET) and EEG, which are correlated to specific processes, makes it possible to model and predict the time course of epileptogenesis and the development of pharmacoresistance. A wide variety of targets for antiepileptic drugs has been identified and can be relevant in relation to pharmacoresistance. The investigations in this thesis, however, were directed towards an in depth study of alterations in functionality and expression of the $GABA_A$ receptor.

1.2 Outline of the thesis

The thesis starts with a general introduction on epilepsy, pharmacoresistance and GABAergic inhibition, including a background on experimental and analytical techniques that have been utilised (**Chapter 2**). Subsequently, the results of the experimental studies performed in this project are divided into three sections, dealing with development of potential biomarkers, changes in expression of the $GABA_A$ receptor and alterations in the efficacy of GABAergic modulators respectively.

In the first section, the first potential biomarker studied was the convulsive threshold as measured by electrical stimulation of the cortex. **Chapter 3** describes whether this threshold can be used as a biomarker for the excitability of the brain in epileptic animals. Surprisingly, this threshold was increased, rather than decreased, after induction of status epilepticus. Furthermore, it is shown that behavioural seizure analysis is a valuable tool in studying mechanisms contributing to epileptogenesis. Next, a method to evaluate disease progression based on the analysis of cortical EEG using event synchronisation was applied to EEG recordings during status epilepticus. The results of this study are described in **Chapter 4**, showing a characteristic four-phasic EEG response during the development of status epilepticus. Moreover, application of this method to a four week EEG recording after induction of status epilepticus indicated that this method is very promising for monitoring and quantifying disease progression and for automatic seizure detection.

The next section deals with possible alterations in the expression of the $GABA_A$ receptor. In **Chapter 5** a novel full saturation method for simultaneous determination of B_{max} and K_D *in vivo* in the rat brain using [^{11}C]labelled flumazenil and PET is introduced. This method is based on analysis of the time course of the $GABA_A$ receptor occupancy following the administration of a saturating dose of flumazenil. It is shown that, by application of population pharmacokinetic (PK) modelling concepts, precise estimates of

both K_D and B_{max} can be obtained. A pertinent feature of the model is that also possible changes in brain distribution of flumazenil (i.e., as result of changes in the functionality of the blood-brain barrier) are accounted for. Subsequently, this full saturation method was used to quantify alterations in expression of the $GABA_A$ receptor in fully kindled rats, as described in **Chapter 6**. In this chapter it is shown that kindling decreased the $GABA_A$ receptor density. Moreover, an alteration in V_{Br} indicates that the blood-brain barrier transport of flumazenil was altered as well.

In the studies described in the last section alterations in functionality and specificity of the $GABA_A$ receptor were investigated. Firstly, the effect of midazolam, as described by an increase in the β-frequency of the EEG, was studied in a post-status epilepticus animal model of epilepsy. The decrease in maximal EEG effect of midazolam after induction of status epilepticus was related to *ex vivo* binding studies using [^{3}H]flumazenil on brain slices of the rats. The results of these studies can be found in **Chapter 7**. The most important observations are that disease progression following status epilepticus was associated with a strongly reduced functionality of the $GABA_A$ receptor, which was only in part accounted for by a reduction in $GABA_A$ receptor expression. In **Chapter 8** the mechanisms of the observed alterations in midazolam effect are investigated in more detail, by performing pharmacokinetic-pharmacodynamic (PK-PD) experiments in epileptic rats *in vivo* using the GABA reuptake inhibitor tiagabine, and the neurosteroid alphaxalone, which is selective for specific subtypes of the $GABA_A$ receptor. Again, the β-frequency of the EEG was used as pharmacodynamic endpoint. The results show that the GABA release and receptor activation remained intact, since the effect of tiagabine was not altered after status epilepticus. Interestingly, however, the efficacy of alphaxalone was increased, pointing to alterations in $GABA_A$ receptor properties, which differentially affect the sensitivity to benzodiazepines and neurosteroids.

Finally, all results described in this thesis are summarised and discussed in the **Summary and general discussion**.