Stereoselectivity in the hepatic metabolism and transport of quaternary drugs of the morphinan type
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In this thesis the potential of some quaternary amine derivatives of the morphinan analogues dextrorphan, levorphanol, dextromethorphan and levomethorphan as model compounds was investigated to study stereoselectivity in hepatic metabolism and transport of cationic drugs. These model compounds possess a rigid stereochemical structure, resulting in major differences in receptor affinity. We assumed that the major influence of the stereochemical structure on the receptor level might also cause differences in carrier-mediated transport and biotransformation processes.

During the development of a reversed-phase HPLC separation system for the analysis of the purity of the quaternized morphinans (N-methyl dextrorphan, N-methyl levorphanol, N-methyl dextromethorphan and N-methyl levomethorphan) it became clear that silanophilic interactions with the stationary phase were involved. Some complicating factors were observed: The separation was sensitive to the brand and type of column material, to slight pH changes of the mobile phase and to column overloading. However, these silanophilic interactions also played a decisive role in the selectivity of the stationary phase, and therefore no attempts were made to suppress the latter. Spherisorb ODS-I stationary phases appeared to be adequate in the separation of the four morphinans and were chosen for the estimation of the purity of synthesized quaternary ammonium morphinans (Chapter 5.1).

For the analysis of biological samples, a C6-bonded stationary phase and a gradient of acetonitrile in 0.02 M sodium phosphate buffer with a low pH as a mobile phase was used. This method was able to separate the respective parent compounds, their primary metabolites and some conjugates in a single run. The silanophilic interactions also caused some troublesome effects in the bioanalysis of the metabolites of the quaternary morphinans. First, the separation was affected by column degradation and by batch-to-batch differences in column material. Small adaptations of the mobile phase composition were made to compensate for small changes in chromatographic performance. Another effect of the involvement of silanophilic interactions was that proteins in the biological samples had a detrimental effect on the column performance. A precolumn switching technique on a reversed phase C8 column with a backflush step to introduce the analytes on the HPLC column was successfully used (Chapter 5.2).

To study the hepatic transport and metabolism, isolated Wistar rat liver perfusions were performed. Experiments with radioactively labelled N-methyl dextrorphan, N-methyl levorphanol, N-methyl dextromethorphan, and N-methyl levomethorphan showed that the initial uptake of the former pair of enantiomers was not stereoselective, but N-methyl dextromethorphan was taken up slightly faster in the liver than N-methyl levomethorphan (Chapter 5.5, and Chapter 5.6 resp.). Further transport steps could not be observed because the compounds were extensively metabolized in a stereoselective way. Samples taken during the liver perfusions were analyzed with the HPLC method described above. Very distinct radiochromatograms were obtained for the enantiomeric pairs. Experiments with selective enzymatic
hydrolysis showed that glucuronides and sulfates of the morphinans were present in the samples. Further identification of the metabolites was accomplished by ionspray LC/MS. Liver perfusions with stable isotope labelled compound were performed and the samples were analyzed with a gradient HPLC method with the same stationary phase but with a volatile mobile phase. Both N-methyl dextorphan and N-methyl levorphanol were found glucuronidated and conjugated with glutathione. In addition, N-methyl levorphanol was hydroxylated and subsequently glucuronidated, and a sulfoconjugate of N-methyl levorphanol was also found (Chapter 5.3). When studying the metabolism of N-methyl dextromethorphan and N-methyl levomethorphan, the O-demethylations of both substances took place at about the same velocity. Further metabolism steps were glucuronidation and glutathione conjugation for the O-demethylated N-methyl dextromethorphan and glucuronidation, glutathione conjugation, sulfoconjugation and hydroxylation for the O-demethylated N-methyl levomethorphan (Chapter 5.4).

The O-demethylation of N-methyl dextromethorphan and N-methyl levomethorphan may be under control of a cytochrome P450 isozyme that is also responsible for the genetic polymorphism of the debrisoquine type. In that case, the O-demethylation of the two enantiomers should be reduced in the livers of Dark Agouti rats, which is an animal model for this genetic polymorphism. We performed isolated rat liver perfusions with livers of these Dark Agouti rats to test this assumption. The O-demethylation of N-methyl dextromethorphan indeed was largely reduced. The O-demethylation of N-methyl levomethorphan, however, was largely unaffected. The velocity of the enzymatic reaction was almost identical to that in Wistar rats. Probably, other isoforms of cytochrome P450 played a role in the O-demethylation of N-methyl levomethorphan. Unfortunately, the Dark Agouti rat did not appear to be a satisfying animal model to study stereoselectivity in other transport phenomena than the initial uptake into the liver of cationic drugs (Chapter 5.7).

Finally, concentrating on the initial uptake of the cationic model compounds, inhibition experiments with procainamide ethobromide and digoxin in isolated rat liver perfusions were performed to characterize the type of carrier system that was involved in the uptake of N-methyl dextorphan and N-methyl levorphanol. It appeared that the uptake in the liver of the enantiomers N-methyl dextorphan and N-methyl levorphanol occurred partly via the type I transport system and partly via the type II or multispecific transport system. The uptake via the type II transport system was found to be stereoselective: The half-life of N-methyl levorphanol appeared to be about 50% larger than that of its enantiomer N-methyl dextorphan (Chapter 5.8).

It is concluded that the quaternary ammonium derivatives of morphinan enantiomers studied provide interesting model compounds for the study of carrier-mediated transport processes for cationic drugs. Their disadvantage for studies with intact organs however is that extensive metabolism occurs at the same time and this largely complicates the study of concomitant transport steps. Therefore, future studies should be performed in isolated plasma membrane vesicles or in reconstituted membrane systems including the transport proteins. Recent studies in our laboratories using expression systems of these proteins, cloned by us and others, indeed demonstrate stereoselectivity in membrane transport of cationic drugs.