SUMMARY

This thesis focuses on some analytical and pharmacological aspects of enantiomers. Enantiomers always exist as a pair of non-identical mirror-images that resemble each other as a left and a right hand. The configuration around the central (carbon) atom is opposite. This remarkable relationship can result in large differences in biological activity between the enantiomers (chapter I.1). Often, one enantiomeric form of a compound is preferred by nature, *e.g.* L-amino acids and D-glucose. The same holds true for various drugs and other biologically active compounds. In many cases, the intended effect resides only in one enantiomer, although in general both are applied (in the racemic drug). Differences can also occur in the disposition of the two enantiomers in the body (chapter I.2). Since in most cases the sum of the two compounds is measured in biofluids, the real pharmacokinetic processes might be obscured.

However, it is rather troublesome to apply and/or to study both enantiomers individually, because of the difficulties of separation for preparative and bioanalytical purposes. The resemblance between the enantiomers is high and they can only be separated in an asymmetric surrounding. A review on appropiate separation techniques, especially chromatography is given in chapter I.3.

In the work presented here enantiomers of oxyphenonium bromide were studied. Oxyphenonium bromide is a quaternary ammonium compound with strong anticholinergic properties. It was known that the enantiomers differ considerably in affinity to the muscarinic receptor. Synthesis, analysis and pharmacology of the enantiomers of this drug were investigated.

The work described in part II was focused on the synthesis of the enantiomers of oxyphenonium bromide. A preparative separation of the enantiomers by crystallization of diastereomeric salts was not successful. Therefore, another approach was used. The acid moiety, cyclohexylphenylglycolic acid (CHPGA), could be resolved by crystallization of its diastereomeric salts with *d*- and *l*-ephedrine. After removal of the amine, the enantiomers of CHPGA were obtained. Enantiomeric purity was assessed with HPLC and DSC. These acid enantiomers were then esterified with β-chloroethyldiethylamine, followed by methylation with methylbromide, resulting in enantiomers of oxyphenonium bromide. The pharmaceutical quality was assessed. Using radioligand binding studies the enantiomers were tested in calf brain preparations with ^{3}H-NMS as the displaced ligand. The dextro-isomer had a

high affinity to the muscarinic receptor, the eudismic ratio being 38. When, instead of methylbromide, ^{14}C- or ^{11}C-labelled methyliodide was used in the methylation step, radioactive enantiomers were obtained. The ^{14}C-labelled material was used for pharmacokinetic experiments in rats. Carbon-11 has a half-life time of 20 minutes and decays under emission of a positron; after labelling with this isotope oxyphenonium could be visualized non-invasively in the body by Positron Emission Tomography.

When a drug has enantiomeric forms, each should be measurable in the same sample. For oxyphenonium bromide, resolution was attempted with chiral HPLC-systems (chapter III.1):

- systems with chiral stationary phases, similar to those that were developed by Pirkle *et al.*
- a system containing *d*-camphorsulphonic acid as a mobile phase component.

Neither approach was successful, probably due to the relatively large distance between the chiral centre and the quaternized nitrogen. So far only the commercially available α_1-acid glycoprotein column (EnantioPac^R) could resolve racemic oxyphenonium bromide. However, efficiency and stability were insufficient for bioanalytical applications.

Therefore, it was tried to resolve the enantiomers of CHPGA, the acid part of the anticholinergic drug (chapter III.2 and III.3). An HPLC system having β-cyclodextrin as the chiral stationary phase was developed. Using aqueous mobile phases, CHPGA and a number of other aromatic carboxylic acids could be resolved. The influence of temperature and sample concentration on selectivity and resolution was taken into consideration, but difficulties in quantitative analysis were encountered. Later on, an opportunity arose to compare the home-made column with a commercially available β-cyclodextrin column. The latter showed better separation characteristics for CHPGA. In addition, a reversed elution order of the enantiomers was seen with the two columns. Enantiomeric impurities of *d*- and *l*-CHPGA were determined as being 0.1 and 0.4 %, respectively.

The differences in affinity of oxyphenonium enantiomers to muscarinic receptors was found to be considerable. The aim of the studies described in part IV was to investigate whether the stereochemical differences also result in unequal pharmacokinetics of the enantiomers. The study was performed in the intact rat and in the isolated perfused rat liver using ^{14}C-labelled material.

In the intact rat the bile duct, urine bladder and carotid artery were cannulated and the lumen of the small intestine was perfused. The radioactivity

in the body fluids was measured during 90 minutes after a bolus injection of *d*- or *l*-oxyphenonium. For *d*-oxyphenonium the total excretion of radioactivity in bile was considerable higher than for *l*-oxyphenonium. However, differences in kinetics were partly obscured by the pharmacodynamic effects of the eutomer, especially after relatively high doses.

In the isolated perfused rat liver, in accordance with the *in vivo* results, the biliary excretion was significantly larger for the dextro- than for the levo-isomer. Hepatic uptake rate was similar, but total biliary excretion of the levo-isomer was only 55 % compared to the excretion of the dextro-isomer. In line with these data, after 2 hours only 30 % of the dose of the dextro-isomer and over 50 % of the dose of the levo-isomer was still found in the liver.

The metabolic profile was investigated using ion pair TLC. In urine only the parent compound was found. In contrast, at least two metabolites were detected in bile for both enantiomers. However, unchanged *l*-oxyphenonium persisted for a longer period at low levels in bile, indicating either a more rapid canalicular transport of the dextro-isomer and/or a more rapid metabolism of *d*-oxyphenonium to cholephilic metabolites.

In part V two clinical experiments are described. In the first, performed in 8 healthy volunteers, an intramuscular dose of 1 mg *d*- or *l*-oxyphenonium bromide was applied in a double-blind cross-over design. Heart rate was observed for 6 hours and urinary excretion for 24 hours. Whereas *d*-oxyphenonium gave a significant increase of the heart rate for at least 3 hours, no significant effect was measured when *l*-oxyphenonium was applied. In 6 out of the 8 volunteers more *d*- than *l*-oxyphenonium was excreted in urine. Unfortunately, so far no plasma concentrations could be measured, which is necessary for a better understanding of the observed differences in urinary excretion between the enantiomers.

In a second study racemic oxyphenonium bromide, its enantiomers and a placebo were inhaled by eight adult patients with chronic airflow obstruction in a four-way double-blind cross-over study. Forced expiratory volume in one second (FEV_1) and slow inspiratory vital capacity (VC) were measured during the first 6 hours after inhalation. *d*-Oxyphenonium was found to be the active isomer, providing better bronchodilation than racemic oxyphenonium. The effect of the distomer on FEV_1 and VC was not significantly different from placebo.

The availability of the enantiomers of oxyphenonium bromide give novel possibilities for the study of muscarinic receptors, of metabolic enzyme systems and of carriers in transport processes of quaternary ammonium compounds.