The question whether ACh sampled by microdialysis reflects adequately the release of the neurotransmitter cannot be fully answered yet. It has been shown that the ACh obtained by microdialysis is completely derived from neuronal activity. Based on in vitro recovery studies, changes of neurotransmitter output can be reliably translated into changes in neurotransmitter concentration in the extracellular fluid. Extracellular fluid concentrations of ACh are assumed to be related to synaptic events, but the mathematical parameters of this relation are unknown at the present time. Several factors can influence the relationship between the release of the ACh and its concentration in the extracellular fluid, e.g. changes in the size of the extracellular space (Lux et al., 1986) and the dynamics of AChE (Weston and Greenfield, 1986). Future experiments, monitoring simultaneously postsynaptic events and extracellular ACh concentrations may further elucidate this relationship between ACh release and the extracellular concentration.

In the light of the present data it is expected that microdialysis of ACh should be a valuable tool for future studies on central cholinergic transmission. In particular, this method can reveal in vivo biochemical effects of potential cholinergic drugs of interest for the treatment of human brain diseases related to the functioning of the cholinergic system. Another interesting possibility of the presented methodology is the combination of microdialysis of ACh and behavioural observations which allows analysis of the neurochemical substrates of behaviour.

6. SUMMARY/SAMENVATTING

Summary

This thesis describes the investigations that have been performed to develop a method which allows the estimation of the in vivo release of acetylcholine (ACh) from the brain of the rat. The microdialysis perfusion technique was chosen as the method for sampling ACh from the striatum of the rat. High performance liquid chromatography (HPLC) was chosen as the analytical method to quantify acetylcholine in the dialysates. Once developed the methodology was used for pharmacological analysis of cholinergic transmission.
The measurement of choline and ACh by means of HPLC, a post-column enzymatical derivatization and electrochemical detection has been simplified and optimised (papers I and II). The separation of these quaternary amines was performed with a cation exchanger and the enzymes were immobilized in an on-line connected reactor. The method appeared to be selective, reproducible and sensitive having a lower limit of detection of 50 fmol. This assay was applied to the measurement of choline and ACh in microdialysates (papers IV, V, VI, VII), blood constituents (paper III) and brain tissue (paper I). In addition, simplified methods for the sample purification of brain tissue and blood constituents have been described. With respect to the analysis of blood constituents it was shown that red blood cells and

plasma do not contain detectable amounts of ACh (detection limit: 10 pmol/ml plasma or red blood cells).
Microdialysis was performed by implantation of a dialysis fibre in the striatum of the rat. When the animal was recovered for at least 18 hours the fibre was perfused with a physiological solution (Ringer) and the outlet of the fibre was connected, by flexible tubing, to the assay system. Samples were automatically injected every 10 or 20 minutes (depending on experimental possibilities) into the HPLC system.
Basal amounts (about 15 fmol/min) of ACh have been measured in rats fitted with a transversal dialysis probe (papers V, VI). In addition to the analytical demonstration of ACh, a further identification of the endogenous ACh was derived by the addition of tetrodotoxin or the deletion of calcium from the perfusion fluid. Both experimental conditions caused a decrease of the ACh in the dialysate to non-detectable levels thereby strongly supporting the neuronal origin of the dialyzed ACh.
A further pharmacological evaluation of the endogenously dialyzed ACh was obtained after administration of the cholinomimetic drug oxotremorine or the anti-cholinergic drug atropine (paper VI). Peripheral administration of oxotremorine induced a 40 % decrease of the output of ACh in the dialysate while atropine increased the output of ACh by about 50%. These results are in accordance with existing theories about the regulation of ACh release.
Two different types of dialysis probes, a U-shaped (papers IV, V, VI, VII) and a transversal one (papers V,VI), have been used. Important differences between the probes were observed: Basal ACh was only measurable when a transversal probe was employed whereas the use of a U-shaped probe required the co-perfusion of small amounts of neostigmine, a reversible acetylcholine esterase inhibitor, in order to prevent the rapid hydrolysis of ACh. The use of a transversal probe resulted in a 20 times higher in vivo recovery of ACh. A possible explanation for these differences is that the distance between the site of ACh release and the dialysis tube is smaller when a transversal probe is employed, thereby increasing the possibility that the released ACh escapes from the action of acetylcholine esterase.
The effects of the voltage dependent potassium channel blocker 4-aminopyridine (4-AP) and its more polar derivative 2,4-diaminopyridine (2,4-DAP) have been investigated on the output of dopamine and ACh as obtained by microdialysis (paper VII). It was shown that both compounds increased the output of ACh when administered intrastriatally. However, only 4-AP increased the output of ACh after peripheral administration. These findings are interpreted as being due to the greater lipid solubility and hence a better penetration into the brain of 4-AP compared to 2,4-DAP. Instrastriatal administration of 4-AP induced a much weaker increase in the output of DA compared to ACh whereas after peripheral administration no change in the output of dopamine was observed. These findings indicate that the sensitivity of excitable membranes to the releasing effects of 4-AP is not the same for all dopamine and ACh containing neurons.
The effects of the anaesthetizing drug gamma-butyrolactone were studied by measuring brain levels of ACh and the microdialysis output of ACh (4.3.3.). It was shown that the microdialysis output decreased dramatically after peripheral administration of the drug while the brain tissue levels were hardly affected. Thus, these two parameters were not related to each other as might have been expected from the paradigm that tissue levels of ACh and the release of ACh are inver-

sely correlated.
Next the microdialysis technique has been used to study the interaction between ACh and dopamine in the striatum (paper VIII, 4.3.4.). After peripheral administration of various cholinergic drugs, oxotremorine, atropine, physostigmine and nicotine, it was shown that the output of dopamine was hardly affected. Conversely, after peripheral administration of the dopamine receptor agonist apomorphine or the dopamine receptor antagonist haloperidol, only minor effects on the output of ACh were observed. These findings suggest, quite unexpectedly, that the release of dopamine and the release of ACh in the striatum are in vivo not thightly coupled.
Finally, the relationship between the neurotransmitter output, as obtained by microdialysis, and the release of the neurotransmitter has been discussed.

Samenvatting

In dit proefschrift wordt de ontwikkeling van een methode beschreven waarmee de afgifte van acetylcholine in de hersenen van een rat kan worden bepaald.
Deze methode kan ons inzicht in het functioneren van hersenenen vergroten. Het kan ook bijdragen tot een beter begrip van de werking van geneesmiddelen in de hersenen.

Men kan de informatie-verwerkende functie van hersenen opvatten als bestaand uit twee typen processen: de voortgeleiding van een signaal (actiepotentiaal) via zenuwcellen (neuronen) en de overdracht van informatie van het ene neuron op het andere door chemische boodschappers (neurotransmitters). Daarbij heeft de voortgeleiding het karakter van een alles of niets proces terwijl de chemische informatie-overdracht tussen zenuwcellen allerlei gradaties kan vertonen die er toe leidt dat andere neuronen juist wel of juist niet geactiveerd worden. Acetylcholine komt ondermeer voor in de uiteinden van uitlopers van zenuwcellen en functioneert daar als neurotransmitter. Na activatie van het betreffende neuron wordt acetylcholine afgegeven door het zenuwuiteinde. Vrijgekomen acetylcholine kan nu gedurende korte tijd de activiteit van andere neuronen beinvloeden. Vervolgens wordt de boodschapper-activiteit van acetylcholine ongedaan gemaakt door een efficiente enzymatische afbraak.

Een van de in vivo methoden om de afgifte van neurotransmitters te bestuderen is de recent door Zweedse onderzoekers (1981) ontwikkelde microdialyse perfusie techniek. Deze methode berust op het uitertst zorgvuldig implanteren van een holle dialyse vezel (doorsnede ca. 0,25 mm) in een specifiek gebied van de hersenen van een proefdier. Nadat het dier hersteld is van de operatie wordt de dialyse vezel aangesloten op een perfusor waarmee langzaam (ca. 5 ul/min) een fysiologische vloeistof door de vezel wordt gepompt. Allerlei kleine biologische moleculen die zich in de hersenvloeistof tussen de zenuwcellen bevinden kunnen nu via de porien in de wand van de vezel naar de vloeistof in de dialyse vezel diffunderen. De met biologische stoffen verrijkte vloeistof, het dialysaat, wordt opgevangen en vervolgens geanalyseerd. Neurotransmitters komen, zij het in zeer geringe hoeveelhe-