EPITHEORESE KLINIKES FARMAKOLOGIAS
KAI FARMAKOKINETIKES, INTERNATIONAL
EDITION 13: 59 (1999)


# Determination of the Neurotransmitter Release in the Central Nervous System *in vivo*

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The determination of neurotransmitters released into a specific brain structure was necessary to define the physiology and the pathophysiology of the CNS functions and also the development of new therapeutic approaches. The first effort to determine the release rates of neurotransmitters in the brain of a lab animal, was by implantation to the brain of a needle, through which normal saline was injected and the same time was withdrawal. Into the samples the concentration of the neurotransmitter was determined. The method named push-pull superfusion and many types of cannulas (parallel needles, concentric needles, close system, open system etc) were been used to have the best results. The main problem of the method was the severe lesion of the area because of the dimensions of the needles and the flow rates of the injected saline, the anesthesia of the animal which influence the specific brain function and also the inability to estimate behaviors the same time.

Because of the above problems a new technique was developed. The main difference of the new technique was that the tip of the cannula was covered by a semipermeable synthetic membrane. Under those circumstances only molecules capable to penetrate the membrane could pass in the superfusate, but this decrease the number of the neurotransmitters for determinations and has a critical delay of molecules recovery. This new technique was named microdialysis and was improved in many ways. The main type of microdialysis probes today is the "upsilon" shaped in which the cannula is consisted by two parallel tubes connected by tubing made by membrane. The superfusion liquid is passing in very slow rates (1-5 µl/min) which could be succeeded by the development of special electronic infusion pumps controlled by microprocessors. On the other hand the undetectable quantities of neurotransmitters, today can be detected with the application of new analytical techniques. The main disadvantages of the method are:

a. The dimensions of the membrane pores, which can forbid big molecules to pass.

b. The long delay the neurotransmitter to penetrate the membrane

c. The membrane material has the ability to attract the glial cells

d. The final concentration of the neurotransmitter in the superfusate is relational low

The recent years there is an effort to develop a system of acute polarometric determination by the insertion in the brain nuclei an ultra thin electrode (in vivo voltammetry). This electrode should have very specific physicochemical attributes concerning its polarity in different concentrations of the neurotransmitter. The last method theoretically is advantageous against the others but in action the success is very limited because of the low specificity of the electrodes.

Today push-pull superfusion technique is improved, by the progress of technology and became really antagonistic to the other brain research methods. Tubes made by neutral, stainless steel have real small dimensions (inner diameter 0,1mm, outer diameter 0,3 mm). The type of the microcannula is concentric, to present the minimal outer diameter in order to minimize the lesion of the tissue and the dimensions of the superfused area. The use of electronic injection pumps permits the control of the CSF flow rates in minimal volumes and in such a way to avoid the nucleus lesion and supedialysis of the extracellular space. To avoid the damage of brain nuclei and at the same time the formation of braid edema the use of electronic peristaltic pumps is necessary. Finally with the combination of the above method with analytical techniques as HPLC, RIA, capillary electrophoresis etc. the released neurotransmitter in the superfusate is easily detectable.

The advantages of the method are:

a. Minimal dimensions of the cannula similar with those of the microdialysis probes

b. The absence of the membrane permits the best output of the neurotransmitter

c. The possibility to use the material of the cannula as monopolar or bipolar electrode for electrical stimulation of the nucleus

d. The ability of application in anesthetized or conscious freely moving animals

e. The possibility of local application of drugs or other neurotransmitters

f. The possibility to use it in acute experiments.

The main disadvantages are:

a) The inability to find the cannula in the free market.

b) The demand of the continuous presence of the researcher and the control of all the volumes injected and withdrawn, mainly in the beginning of the experiment, so as to avoid the obstruction of the withdrawal tube.