Disertation work brings new findings in the field of biochemistry and analysis of neuroactive steroids. The publications involved to the dissertation work deal with biochemical analysis of neuroactive steroids and its application in biomedicine.

Some endogenous steroids can act as efficient neuromodulators. These substances are known as neuroactive steroids. If they are synthesized directly in the central nervous system, they have been named the neurosteroids (Schreiber 1980; Corpechot, Robel et al. 1981). Some of the neuroactive steroids act as neuroactivators, the others are neuroinhibitors (Kimonides, Khatibi et al. 1998; Kaasik, Kalda et al. 2001; Lockhart, Warner et al. 2002). The neuroactive steroids operate usually as modulators of membrane receptors (GABA-receptors and NMDA-receptors) responsible for the permeability of ion channels in neuronal cells.

Generally, while physiologic functions and pharmacokinetics of endogenous neuroactive steroids studied on animals was investigated (Wang, Wahlstrom et al. 1997; Norberg, Backstrom et al. 1999), the number of studies concerning neuroactive steroids in human body fluids and tissues in connection with physiology is insufficient (Bicikova, Tallova et al. 2000; Strohle, Romeo et al. 2003). The main

problem is probably laboriousness of corresponding analytical methods for substances which are efficient from picomolar concentrations. Availability of such methods could facilitate the utilization of neuroactive steroids in clinical praxis and accelerate the research of the role of neuroactive steroids both in human physiology and in pharmacokinetics.

The disertation work offers several original high sensitive and selective methods of simultaneous determination of neuroactive steroids including their sulfates. The methanolysis of sulfates of pregnanolone isomers in human serum was described (Havlikova, Hill et al. 2006). This method uses the solution of trimethylchlorsilane in methanol for solvolysis of sulfate group. Furthermore, the original GC-MS method for determination of its free analogs was modified (Havlikova, Hill et al. 2006). Above mentioned methanolysis was used for determination of 17-hydroxypregnenolone sulfate (Vcelakova, Hill et al. 2007). This part of disertation work targets the metabolism and diagnostic application of this not commonly studied steroid. During the fellowship in the Department of Physiology and Molecular Biology, University College London, the analysis of neurosteroids in male rat brain were performed. Using the careful proceeding for preparation of sample followed by GC-MS analysis, the presence of steroids and their sulfates in rat brain was described (Ebner, Corol et al.

2006). Beside these findings, the relationship of well known neuroactive steroid pregnenolone sulfate to less common antiandrogen epitestosterone was discussed (Havlikova, Hill et al. 2002).

Investigation of physiology of rarely determined steroids has brought number of new observations as was evidence of antiandrogenic action of epitestosterone or findings about the inhibition of some steroidogenic enzymes. Concerning neuroactive steroids, in consequence to the disertation work, some important publications describing the action of neuroactive pregnanolone isomers during pregnancy and arround parturition were published (Klak, Hill et al. 2003; Parizek, Hill et al. 2005; Hill, Cibula et al. 2007; Kancheva, Hill et al. 2007). These publications report strong changes in activity of enzymes regulating the ratio of neurostimulatory and neroinhibitory steroids. These works answer the questions concerning the role of steroidogenesis in the mechanism of initiation of human delivery. Finally, the precise evaluation of age relations and sex differences in serum levels of 17-hydroxypregnenolone sulfate and pregnenolone sulfate represents important findings in clinical biochemistry and endocrinology.