ABSTRACT

This doctoral thesis was focused on the multidisciplinary characterizatrion of interactions between hormones and their receptors during sperm capacitation, which is the final step in sperm maturation in mammals and the key event to achieve egg fertilization. Three oestrogenic hormones (17β-estradiol (E2), 17α-estradiol (αE2), 17α-ethynylestradiol (EE2)), which are part of environmental pollution, were selected for the study. These hormones are called endocrine disruptors as they can cause disorders of the hormonal system in animals, thereby adversely affecting physiological processes, including reproduction. A new analytical HPLC-MS/MS method was developed for monitoring of concentration changes of oestrogens (E2, αE2 and EE2) during time-dependent capacitation of mouse and rat sperm *in vitro*. For individual oestrogens, the concentration dependences of free, unbound hormone on the capacitation time were measured for three concentrations of individual hormones (200, 20 and 2 μg/L). The obtained concentration data were converted into values of relative concentrations, which were subjected to kinetic analysis. Kinetic models were proposed for the interaction of individual hormones with sperm, which were described by a system of differential equations with optimization of rate constants and molar ratio. The optimization was performed on the basis of searching the minimum of absolute values of the difference between the theoretical and experimentally obtained values of the relative concentration B_t. The optimized constants were used to calculate the theoretical $B(t)$ curves and a good agreement between the measured and calculated relative concentration values was obtained. From a kinetic point of view, a similar trend was observed for all three studied hormones, assuming an autocatalytic reaction between the hormone and the cytoplasmatic receptors of sperm to form an unstable adduct, which subsequently decomposes. However, the kinetic scheme is proposed to differ in transportation of individual hormones across the sperm cytoplasmic membrane. The results obtained in this doctoral thesis showed that kinetic analysis has the potential to be used as a tool for monitoring and predicting specific molecular mechanisms involved in cell signaling in a wide range of physiological and pathological processes.