Ischemia of heart and brain tissue is one of the most common causes of death worldwide. During the last years, it was shown that many proteins called potassium channels are localized in the membrane of mitochondria. Similar proteins were identified also in the plasma membrane. In a number of studies was shown that opening of potassium channels by pharmacological substances, called potassium channel openers could initiate processes, which protect mitochondria against damage caused by various factors including ischemia/reperfusion. Unfortunately, mechanisms of this process are still unknown.

Large-conductance calcium-activated potassium channel is one the channels identified both in the plasma membrane and in the inner mitochondrial membrane. The activity of these channels is inhibited by heme or its oxidized form hemin. Heme is an extremely important molecule for the proper functioning of our organism and it is present, among others, in hemoglobin where is responsible for oxygen binding and its transportation to the cells. Heme, besides the oxygen, could also bind other molecules such as carbon monoxide and hydrogen sulfide. For many years, both molecules were recognized only as toxic gases. However, in recent years, it was shown that when produced in small amounts within cells, they play a beneficial role in many processes in our organism. The latest research, showed that carbon monoxide and hydrogen sulfide could interact with potassium channels resulting in their opening, what in turn causes protective effects for the cells against damage caused by various factors, including ischemia – a phenomenon called cytoprotection.

It was shown that heme/hemin causes inhibition of the activity of large-conductance calcium-activated potassium channel by binding to a site located inside the mitochondria. My preliminary results showed that hemin inhibits the channel also from the external side of mitochondria. It has never been described before. Hemin action from the opposite side points to the existence of a new heme/hemin binding site in the large-conductance calcium-activated mitochondrial potassium channel. Therefore, the main aim of this project is to identify and characterize this new heme binding site. The studies will be carried out using the sophisticated electrophysiological technique – patch-clamp. The patch-clamp technique allows for the recording of the activity of single channel molecules. To this end, I will isolate mitochondria from cells. Since mitochondria contain two membranes from which only the inner membrane contains channels under study, the mitochondria will undergo swelling to obtain mitoplasts - objects that lack the outer membrane. Mitoplast will be used directly in patch-clamp experiments. It is known that certain amino acids tend to be found in heme binding motifs. This includes particularly cysteines and histidines. To find out which cysteines and histidines are responsible for hem/hemin binding I will carry out site-directed mutagenesis and substitute external cysteines and histidines with amino acids of different properties. By testing heme/hemin inhibition of such mutants I will be able to pinpoint to the new heme/hemin binding site at the external side of the channel.

Heme, which is bound in the heme binding motif, could be a physiological mediator for gasotransmitters interaction with the channel, and in turn could be intermediary in cytoprotection mechanisms. The mechanism under my investigation may, in the future, contribute to the development of new therapies against ischemia of heart and brain tissue.