ABSTRACT

The extract from the plant *Galega officinalis* containing the guanidine derivative galegin has been used in the treatment of diabetes-associated complications since middle ages. Nevertheless, the positive effects of the treatment were often overweight by the adverse side effects. Some sixty years ago guanidin was replaced by the less toxic synthetic biguanide derivatives – metformin, phenformin and buformin, the latter two being withdrawn due to the unacceptable risk of fatal lactate acidosis. Metformin is still widely used antidiabetics and belongs to the first choice drugs in the treatment of type 2 diabetes. Phenformin is now gaining renewed attention with regard to its antineoplastic properties.

Despite its long-term clinical use the mechanism of biguanides action is not fully understood yet. At present it is generally accepted that the core of its antihyperglycemic effect lays in the inhibition of hepatic gluconeogenesis. In contrast, there is less consensus regarding the particular metabolic pathway or target that are responsible for the metformin-induced attenuation of gluconeogenesis. For a long time, a hot candidate for metformin target in the cell was AMPK (AMP-activated kinase) but the metformin effect was proved also in mice carrying the dominant negative mutation of AMPK α subunit. Quite recently, a study identifying mitochondrial glycerol-3-phosphate dehydrogenase as the new metformin target has been published. Numerous reports demonstrate also the metformin effect on non-enzymatic processes including membrane fluidity or MPTP (mitochondrial permeability transition pore) sensitivity to calcium.

The common feature of biguanide action that reconciles above mentioned hypothesis is their effect on the cell energy metabolism. Therefore, the main aim of the presented thesis is to study the effect of biguanides (metformin and phenformin) on the function of isolated liver mitochondria *in vitro* and on the energy metabolism in the liver *in vivo.* The submitted thesis is based on three papers, two of them has been already published (Publication A, B) and one is now under review (Publication C).

Our data showed that both liver homogenate and isolated liver mitochondria represent comparable models for the study of effect of metformin action *in vitro.* Liver homogenate is particularly suitable model in experiments that require longer incubation of the tested substance with the mitochondria.

We further found that *in vitro* metformin selectively inhibits the activity of complex I (EC_{50} = 5 mM) and that it does not affect the activities of other components of mitochondrial respiratory chain. The metformin-induced partial inhibition of complex I may be compensated by the increased supply of electrons via complex II. The mechanism of action of metformin and

phenformin shares a lot of common features. Both compounds inhibit mitochondrial respiration of NADH-dependent substrates in dose-dependent manner, phenformin being more effective (EC_{50} = 0.25 mM) compared with metformin. We were first to demonstrate that in high doses phenformin inhibits also complex II and complex IV and in contrast to metformin, phenformin-induced inhibition of respiration could not be fully compensated by electron supply via complex II. Both biguanides increase the resistance of MPTP to calcium cations. Both metformin and phenformin action is not dependent on the cell integrity and could be demonstrated in the liver homogenate as well as in permeabilized hepatocytes.

The persistent stumbling block in the research focused on the mechanism biguanide action is the discrepancy between the effective concentration *in vitro* and real concentrations found in serum *in vivo*. We brought the evidence that long-term metformin administration *in vivo* results in comparable changes in mitochondrial metabolism as acute metformin administration *in vitro* – decreased respiration of NADH-dependent substrates and lowered activities of some mitochondrial enzymes. In accordance with these findings we observed the decreased ATP re-synthesis during reperfusion in the liver after short-term ischemia in rats administered metformin for 10 weeks. Our data support the hypothesis that *in vivo* metformin accumulates within mitochondria where it reaches sufficient effective concentration.

We demonstrated protective effect of metformin against oxidative stress after ischemia/ reperfusion injury, the effect being more pronounced in fatty liver. At the same time we showed the decreased reactive oxygen species formation in submitochondrial particles *in vitro* in metformin-treated group.

Our data support the hypothesis that the metformin effect is pleiotropic and involves more partial processes rather than the one unique target theory. Such a complex biguanide action provides better platform for explanation of their multiple and diverse effects.