Abstract

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Title of Doctoral Thesis:	The effect of polyphenolic substances on vascular smooth muscle

Polyphenolic substances are one of the most abundant secondary plant metabolites. Many substances from simple phenols with one benzene core to the polymeric compounds belong to these groups. Two groups, coumarins and flavonoids, were selected for this dissertation. The aim of this thesis was to analyse their effect on vascular smooth muscle in *in vitro* and *in vivo* conditions. In the case of coumarins, the aim was reached by writing of a review article. The effect of flavonoids, including their metabolites was experimentaly tested and some of the results have already been published.

The first step in my research was to perform a screening of metabolites of quercetin, the most occuring flavonol in the human diet, and compare their vasorelaxant potential. Experiments were performed on isolated rat thoracic aorta. During the testing, the most effective metabolite was definitely 3-(3-hydroxyphenyl)propionic acid (3-HPPA), which was at least one order more potent than quercetin and others metabolites (espetially

3,4 dihydroxyphenylacetic acid and 4-methylcatechol). Comparing of the effect of these metabolites on a resistant vessel *(arteria mesenteric*a) showed significant differences, where 3-HPPA was on the contrary less active than other two metabolites. In another part of *in vitro* study, the mechanism of action was tested (vessels without endothelium, an ainhibitor of NO synthesis or a muscarinic receptor antagonist). Even these results pointed to different mechanisms of action, in particular when comparing 3-HPPA and 4-methycatechol.

In vivo experiments were firstly carried out on Wistar:Han rats, in which the changes of arterial blood pressure and heart rate after the application of above metioned metabolites were monitored. The significant decreases in systolic and diastolic pressures were showed after the application of doses of 10 mg/kg and higher. Antihypertensive effects were also confirmed on rats with pathologicaly elevated blood pressure (spontaneously hypertensive rats, SHR). Due to the short lasting effect of these doses and immediate normalization of blood pressure, we approched to the simulation of long lasting absorption of metabolite from colon by application of slow i.v. infusion. Also in this case we found out dose-dependent effects on blood pressure, but only at the highest concentration (5 mg/kg/min for 5 minutes) the statisticaly significant decrease comparing to the control was observed. The effect was again relativelly short-lasting. At the same time, we excluded, that the decrease of blood pressure was caused by a depresive effect on the heart and so we confirmed the effect on vascular smooth muscle *in vivo*.

In vivo we also tested quercetin-3-O-glucuronide, which was uneffective under *in vitro* conditions and of which a different research group speculated that it is a carrier of active substance (quercetin) and thus also responsible for the *in vivo* decrease in blood pressure after oral quercetin administration. Regarding to our results, we consider this theory as unlikely.

Part of this thesis is *in vivo* testing of a specially modified quercetin for i.v. administration in saline. Regarding to the quercetin lipophility, we were the first group, which could analyse the *in vivo* effect of quercetin without addition of organic solvents. Quercetin decreased the blood pressure immeditely and also later during the first five hours after administration.

In conclusion, we proved our initial theory, thus some of quercetin metabolites, created by metabolism in the colon, have vasorelaxant potential and therefore are able to affect blood pressure.