ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové	
Training Workplace	Department of Pharmacology and Toxicology
Doctoral Degree Program	Pharmacology and Toxicology
Candidate	Patrícia Alexandra Alves Dias, M.Sc.
Supervisor	prof. Přemysl Mladěnka, Pharm.D., Ph.D.
Advisor	assoc. prof. Jana Pourová, Pharm.D., Ph.D.
Title of Doctoral Thesis	Study of the mechanisms of action of phenolic compounds on the vascular smooth muscle

Cardiovascular diseases including hypertension, coronary artery disease, peripheral artery disease, and cerebrovascular disease remain the leading cause of death worldwide. In addition, discouraging estimations have suggested a future increase in the number of cardiovascular patients. Thus, novel treatment modalities are clearly needed to prevent or reverse these epidemic trends.
Phenolic compounds contain one or more hydroxyl groups bound to a benzene ring. This class of chemicals includes: a) natural compounds (e.g., dietary polyphenols and small phenolic metabolites) referred to as nutraceuticals due to their claimed health-promoting effects and b) synthetic compounds (e.g., bisphenols) which, on the contrary, have been suggested to negatively affect human health.

Even if there are claims that polyphenol-rich diet is associated with cardioprotective effects, important questions remain to be elucidated. In particular, the parent compounds have mostly low bioavailability, so the bioactivities have been ascribed to their colonic metabolites. 3-hydroxyphenylacetic acid (3-HPAA) is a small phenolic metabolite that has previously shown vasorelaxant effects ex vivo. Hence, the first part of this dissertation aimed to investigate whether 3-HPAA exerts haemodynamic effects in vivo as well and to elucidate the mechanism of action. The intravenous administration of 3-HPAA as both a bolus and infusion decreased systolic and diastolic arterial blood pressure in spontaneously hypertensive rats. The blood pressure-decreasing effect was dose-dependent and was not accompanied by significant changes in heart rate. Additional ex vivo isometric tension recordings revealed that 3-HPAA relaxes the isolated porcine coronary artery, which was selected as a model for determination of the mechanism of action at the molecular level. The vasorelaxant effect was partially dependent on the integrity of the endothelial layer and was significantly decreased after endothelial nitric oxide synthase (eNOS) inhibition. On the contrary, the inhibition of small and intermediate conductance calcium-activated potassium channels (SKCa and IKCa), cyclooxygenases, or L-type calcium channels as well as antagonism at muscarinic receptors had no impact on 3-HPAA-induced vasorelaxation. In summary, the findings suggested that 3-HPAA decreased blood pressure in vivo through peripheral vasorelaxation via a mechanism likely involving nitric oxide release by the endothelial layer.

Bisphenols are endocrine-disrupting chemicals widely used by the industry in the production of polycarbonate plastic and epoxy resins in food packaging materials, beverage cans, thermal receipts, electronic devices, etc. Since the lead compound, bisphenol A (BPA), has been inculpated with many harmful effects on human health, it has been replaced by novel, so-called next-generation (NextGen) bisphenols. These alternative compounds are, however, much less studied and are now pervasive throughout the environment. Therefore, in the second part of this dissertation, we aimed firstly to systematically review the literature available on bisphenols and their potential impact on the cardiovascular system. Due to being present in many daily use products, humans are inevitably exposed to bisphenols. Indeed, bisphenols have been detected in

different human biological samples. Reported total serum levels of BPA (i.e., including the conjugated metabolite) were up to ~ 430 nM, whereas those of free BPA were up to 80 nM. Although reports on the levels of NextGen bisphenols are scarcer, the number of studies has been increasing. For example, the maximum serum levels of bisphenol S reported were ~ 680 nM. In vitro studies showed that bisphenols interact with ion channels, thyroid, oestrogenic and androgenic receptors. In the case of BPA, vasodilatory effects were shown ex vivo, while an unexpected increase in arterial blood pressure occurred in vivo and in observatory cross-sectional human studies. Additional negative effects have been described on hepatic lipid and glucose metabolism and coronary artery disease. However, due to inconsistencies and even contradictory findings, there is a need for novel studies, particularly focusing on the newly introduced NextGen bisphenols. For this reason, the next stage of this PhD project centred on testing 14 bisphenols (bisphenols A, AF, AP, B, BP, C, E, F, G, M, P, PH, S and Z) and comparing their effects in vitro (human and rat cell lines), ex vivo (isolated rat aorta) and in vivo (Wistar Han rats, acutely or chronically exposed to either low environmental or high toxic doses). Eight of the tested bisphenols relaxed the rat aorta with different potencies. Bisphenol AF (BPAF) was the most potent vasodilator, with an EC50 of 57 μM, and the mechanism of action seemed to be based on the blockade of L-type calcium channels. The cytotoxicity of bisphenols towards 4 human and rat cell lines (H9c2, A-10, MCF7/S0.5 and MCF7/182R-6) showed variable potencies ranging from micromolar units to millimolar concentrations. Hence, changes in arterial blood pressure and cardiotoxicity could occur. However, the in vivo acute effects of three doses (0.005, 0.05 and 2.5 mg/kg) of BPAF and 3 other analogues (bisphenols A, S and F) on the cardiovascular system were rather biologically negligible. BPAF was also administered chronically at a dose of 2.5 mg/kg daily for 4 weeks to normotensive Wistar Han rats, but there were no changes in arterial blood pressure. In summary, although bisphenols can relax vascular smooth muscles, the effective concentrations are too high to produce clear cardiovascular effects in relation to common biological exposure.Následuje překlad abstraktu práce do anglického jazyka