Regulation of Vascular Tone by Plant Polyphenols: Role of Nitric Oxide

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Abstract. Vascular endothelium plays a crucial role in regulating blood flow and vascular tone It can synthesize and release different relaxant factors including nitric oxide (NO) This article summarizes pharmacological properties of red wine polyphenol extracts (RWPC) with respect to endothelial NO It is shown that RWPC produces endothelium-dependent relaxation as a result of enhanced NO synthesis rather than enhanced biological activity of NO or protection against breakdown by O^{2-} The mechanisms involve influx of Ca^{2+} and production of O^{2-} within the endothelial cells These results suggest that RWPC, by releasing endothelial NO, may have therapeutically relevant effects against cardiovascular diseases

Introduction

Vascular endothelium lies at the interface between the circulating blood cells and the vascular smooth muscle cells, responds to flow and shear stress and vasoactive stimuli It plays a crucial role in regulating blood flow and vascular tone as a consequence of the synthesize and release of different relaxant factors such as nitric oxide (NO), prostanoid derivatives and the so called endothelium-derived hyperpolarizing factor (Furchgott and Vanhoutte 1989) The release of these substances produces endothelium-dependent vasorelaxation of different vascular beds from different species In many vascular pathologies, such as hypertension, diabetes and atherosclerosis, endothelium-dependent vasorelaxation to different vasodilator agonists is reduced One of the mechanisms accounting for this dysfunction of the endothelium is a decreased release of NO Thus, endothelial NO plays an important role in regulating vascular tone both in humans and animals under physiological and pathophysiological status

Apart from physiological stimuli, very recently it has been reported that many plants, including grapes, known to contain polyphenols can induce endothelium-dependent vasorelaxation probably via NO release, or enhanced biological activity of NO, leading to an elevated accumulation of guanosine 3', 5'-cyclic monophosphate (cyclic GMP) (Fitzpatrick *et al* 1993) This property might be involved in the protective effects of polyphenols

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against cardiovascular diseases in addition, to their antioxidant and free radical scavenger actions Also, this pharmacological effect of polyphenols is of importance, because NO has been shown not only to have vasorelaxant and antiaggregatory properties Also, NO is able to limit the flux of atherogenic plasma proteins into artery wall

This article sumarizes our recent results regarding the mechanism by which plant polyphenols, especially red wine polyphenol extracts (RWPC) mediate their vascular effect, with respect to endothelial NO The pharmacological approaches used to study the effect of WPC comprise both functional and biochemical experiments in isolated blood vessels and in cultured endothelial cells (Andriantsitohaina *et al* 1998) The first part concerns functional and biochemical results on vascular tissues (Andriambeloson *et al* 1997a) The second part deals with the cellular mechanism by which plant polyphenols might produce NO (Andriambeloson *et al* 1997b, 1998a)

Materials and Methods

Aortic preparation and mounting

Male Wistar rats (12 14 weeks old) bred in our institute from genitors provided by Iffa Credo (Abresle, France) were used The thoracic aorta was removed and carefully cleaned of adhering fat and connective tissue, and cut into rings (2–3 mm length) The rings were then mounted in standard organ baths Resting tension was adjusted to 2 g Tension was measured with an isometric force transducer

In some experiments, the endothelium was removed by gently rubbing the intima surface with curved forceps The presence of functional endothelium was assessed in all preparations by determining the ability of acetylcholine (1 μmol/l) to induce more than 50% relaxation of rings pre-contracted with noradrenaline (1 μmol/l)

Cultured bovine aortic endothelial cells

Endothelial cells were isolated from bovine aortae (BAEC) as previously described (Kessler and Lugnier 1995) Freshly excised bovine thoracic aortae were placed in ice-cold Ca^{2+}- and Mg^{2+}-free salt solution Aortae were washed abundantly with Ca^{2+}- and Mg^{2+}-free salt solution and the endothelial cells were harvested by lightly scraping the intimal surface with a scalpel blade

After further dissociation by collagenase treatment (120 U/ml collagenase D) and centrifugation, the pellet was resuspended in culture medium (50% Dulbecco modified Eagle's medium and 50% Ham's F12 medium) supplemented with heat-inactivated fetal calf serum, L-glutamine, heparine, penicillin, streptomycin, vitamin C and amino acids Endothelial cells were then subcultured in plastic flasks maintained at 37 °C in a 5% CO2 humidified incubator

Results and Discussion

It has been found that RWPC produces an endothelium-dependent relaxation of rat aorta at low concentrations ($< 10^{-2}$ g/l) which may well occur in human or animals in vivo (Andriambeloson *et al* 1997a) This effect results from enhanced synthesis of NO rather than enhanced biological activity of NO or protection against breakdown by superoxide anions (O^{2-})

The mechanisms by which RWPC induced endothelial-dependent relaxation have been investigated in rat thoracic aorta rings with endothelium (Andriambeloson *et al*

1998a) The results suggest that RWPC produces endothelial-NO derived vasorelaxation through an extracellular Ca^{2+}-dependent mechanism They also show that B pertussis toxin- or cholera toxin-sensitive G-proteins, activation of phospholipase C or phospholipase A2 pathways, protein kinase C or tyrosine kinase may not be involved

The mechanisms by which RWPC generates NO have been investigated in BAEC in respect to calcium handling (Andriambeloson *et al* 1997b) The results suggest that generation of NO produced by RWPC requires influx of Ca^{2+} and involves the production of O^{2-} in BAEC They also indicate like in the aorta that RWPC may not mediate its action through G proteins sensitive to cholera and B pertussis toxins or through the stimulation of either phospholipase C or phospholipase A2 pathways

Finally, the possible active principles which support the endothelial NO-dependent relaxation produced by RWPC have been investigated in rat aorta (Andriambeloson *et al* 1998b) The results show that the chromatographically resolved fractions enriched into anthocyanins and those enriched into oligomeric condensed tannins exhibit a pharmacological profile comparable to the original RWPC The latter coumpounds may contain the active(s) principle(s) for RWPC-induced endothelial-NO vasorelaxation

In conlusion, it can be suggested that one of the mechanisms of the cardioprotective effect of RWPC may be the increase of endothelial NO production Therefore, some of the polyphenols present in red wine may produce therapeutically relevant effects by knowing the important role of NO in regulating vascular tone both in humans and animals under physiological and pathophysiological status

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