The effects of lectins on the release of EDRF from rabbit aorta

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The effects of the lectins, wheat germ agglutinin (WGA) and concanavalin A (Con A) on endothelium intact pre-contracted rabbit aorta were investigated. WGA (8.3 μM) produced endothelium-dependent relaxations, while Con A (4.3 μM) produced a partially endothelium-dependent relaxation. The endothelium-dependent relaxations to WGA were completely reversed by N-acetylglucosamine, haemoglobin and methylene blue and were partially reversed by N^G-monomethyl-L-arginine (L-NMMA). It is suggested that WGA works as an agonist in releasing endothelium-derived relaxing factor (EDRF) from the endothelium of rabbit aorta by interacting with a glycosylated receptor on endothelial cells.

Keywords: Wheat germ agglutinin; concanavalin A; EDRF; rabbit aorta; N-acetylglucosamine; haemoglobin; methylene blue; endothelial cells

Introduction Lectins have been used to characterize and isolate cell surface glycoproteins for many cell types including endothelial cells (Hedo, 1984). Lectins have the ability to bind to specific saccharide groups of glycoproteins and this binding can be reversed and blocked by specific sugars (Grulich-Henn *et al.*, 1988). Some lectins have been used to label specifically the vascular endothelial cells without labelling the underlying smooth muscle cells (Raub *et al.*, 1990).

One of the many functions of the endothelium is the mediation of vasodilatation, which involves the release of a potent vasodilator referred to as endothelium-derived relaxing factor (EDRF) (Furchgott, 1984). EDRF is very labile with rapid biological action. The responses to EDRF are similar to the responses of nitrovasodilators in that they both stimulate the enzyme guanylate cyclase increasing cellular levels of guanosine 3': 5'-cyclic monophosphate (cyclic GMP) leading to vascular smooth muscle relaxation (Furchgott, 1984). Endothelium-dependent vasodilators also stimulate the endothelium to release prostacyclin (PGI₂). Investigators have suggested that in cultured endothelial cells the release of EDRF and PGI₂ is coupled (De Nucci *et al.*, 1988).

Thrombin stimulates synthesis and release of PGI₂ from human umbilical vein endothelial cells (Weksler *et al.*, 1978) and in these cells WGA has been demonstrated to block thrombin-induced increase in intracellular free calcium, [³H]-arachidonate release, and PGI₂ release (Grulich-Henn *et al.*, 1987). We have shown that WGA does not block the thrombin, bradykinin, or acetylcholine receptor on the native endothelium but binds to its own receptor and causes the release of EDRF which relaxes vascular smooth muscle.

Methods Male New Zealand White rabbits were anaesthetized with sodium pentobarbitone. The aorta was removed and placed in cooled physiological salt solution (PSS) of the following composition (mM): NaCl, 140, KCl 5, CaCl₂ 1.5, MgCl₂ 1, D-glucose 10, HEPES/Na HEPES 5, pH 7.4 plus EDTA 10 μM at 37°C. Rings of approximately 3 mm in width were used with the endothelial layer intact along with endothelial denuded tissues. The endothelium was removed by gently rubbing the intimal surface of the blood vessel with a wooden toothpick. The tissues were set up under 1 g of tension in PSS aerated with room air at 37°C; the tension was adjusted during the first 2 h of equilibration. The tension was measured with an isometric force transducer (Grass FT03C) and displayed on a polygraph (Beckman R-611). The tissues

were contracted with 80 mM KCl to determine the maximum tension of the tissue. The presence of endothelium was determined by the addition of acetylcholine (ACh), 300 nM, to pre-contracted tissues.

The effects of the different lectins were tested on the tissues contracted with phenylephrine (300 nM PhE on endothelial intact tissues and 100 nM PhE on tissues with the endothelium removed).

Results The effects of WGA and Con A were tested up to 8.3 μM and 4.3 μM , respectively. The effect of WGA in the presence and absence of endothelium is shown in Figure 1a and b. WGA was added to endothelium-intact and endothelium-denuded tissues starting at 1 $\mu\text{g ml}^{-1}$ (0.028 μM) and ending at 300 $\mu\text{g ml}^{-1}$ (8.3 μM). WGA caused an endothelium-dependent relaxation with a half maximum inhibitory concentration (IC₅₀) of $0.9 \pm 0.2 \mu\text{M}$ ($n = 6$). No relaxation was measured when WGA was added to the endothelium-denuded tissues even at high concentrations of 8.3 μM WGA.

Con A caused both endothelium-intact and endothelium-denuded tissue to relax after a Phe sustained contraction. At higher concentrations (4.3 μM), Con A caused an $80.1 \pm 3.5\%$ ($n = 6$) relaxation in the presence of endothelium and a $21.0 \pm 3.6\%$ ($n = 6$) relaxation in the absence of endothelium.

In tissues pretreated with atropine (1 μM), WGA (8.3 μM) was equally effective in causing relaxation of precontracted endothelium-intact aortic rings, suggesting that WGA is not working through the acetylcholine receptor. Also in tissues relaxed with WGA (1 μM , approximately 50% relaxation), thrombin (0.5 u ml^{-1}), bradykinin (10 nM) or acetylcholine (300 nM) were able to relax the tissue further without any apparent inhibition of the response.

The effects of various interventions on the relaxation to WGA are shown in Figure 2. These interventions demonstrate that the relaxation to WGA is due to the release of EDRF from the endothelium. In 6 experiments, haemoglobin (10 μM), caused a $108 \pm 5\%$ reversal of the WGA-induced relaxation (Figure 2c). Methylene blue (10 μM) caused a $112 \pm 3\%$ reversal of the WGA-induced relaxation (Figure 2d). The guanylate cyclase inhibitor LY83583 (6-anilino-5,8-quinoline-dione) at a concentration of 3 μM caused a $95 \pm 4\%$ reversal of the WGA-induced relaxation (Figure 2e). The inhibitor of EDRF synthesis, N^G-monomethyl-L-arginine (L-NMMA) at 1 mM attenuated the response to WGA by $55 \pm 2\%$ (Figure 2f). N-acetyl glucosamine (20 mM) caused a $99 \pm 3\%$ reversal of the WGA-induced relaxation (Figure 2a). The effect of the sugar, N-acetyl glucosamine, was specific because after

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Figure 1 (a) Representative isometric tension recordings of paired rabbit aortic rings with endothelium (+E) and without endothelium (-E) contracted with phenylephrine (PhE) showing endothelium-dependent relaxations to wheat germ agglutinin (WGA) added cumulatively. (b) Effect of increasing concentrations of WGA on the contractile responses of rabbit isolated aortic rings. Responses to WGA with and without endothelium were investigated in tissues from the same animal. Data are expressed as percentage relaxation of the response to phenylephrine (300 nM PhE with endothelium and 100 nM without endothelium), and shown as mean ($n = 6$) with s.e.mean indicated by vertical bars.

reversal of the WGA-induced relaxation, acetylcholine (300 nM) could still produce 100% relaxation ($n = 6$).

Discussion In endothelium-intact rabbit aorta contracted with PhE (<50% max.) WGA and Con A relaxed the blood vessel. The effect of Con A was partially endothelium-

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Figure 2 Representative isometric tension recordings of rabbit aorta with the endothelium intact showing the effects of various interventions on the relaxation to wheat germ agglutinin (WGA). The effects of N-acetyl glucosamine (GluNAc) at 20 mM on the effects of 8.3 μM WGA is shown in (a). The effect of 10 μM haemoglobin (Hb) (b), 10 μM methylene blue (MeB) (c) and 10 μM LY83583 (d) on the relaxation to WGA are shown. The relaxation response to WGA in the presence of 1 mM N^G -monomethyl-L-arginine (L-NMMA) is shown in (e). All tissues precontracted with phenylephrine (PhE).

dependent, whereas the response to WGA required a functionally intact endothelium. The endothelium-dependent relaxation to WGA was concentration-dependent with an IC_{50} of $0.9 \pm 0.2 \mu\text{M}$. L-NMMA attenuate the endothelium-dependent vascular relaxations induced by various EDRF agonists (Johns *et al.*, 1990). Relaxations induced by WGA were attenuated by L-NMMA and completely inhibited by methylene blue, haemoglobin (Martin *et al.*, 1984), and LY83583 (Mulsch *et al.*, 1988). The effect of WGA is therefore consistent with many other endothelium-dependent vasodilators and suggests the relaxation is due to the release of EDRF (Furchgott, 1984). The relaxation to WGA was reversed by N-acetyl glucosamine which is specific for WGA recognition (Grulich-Henn *et al.*, 1988). This sugar was ineffective against acetylcholine-induced relaxation. It is concluded that the endothelium 'recognizes' WGA as an agonist and the specific interaction with the vascular endothelium results in the release of EDRF. Whether the interaction of WGA is with a lectin receptor or with the glycosylation site on another receptor has yet to be determined.

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