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***Arbutus unedo* Induces Endothelium-Dependent Relaxation of the Rat Isolated Aorta**

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ABSTRACT

Arbutus unedo L. (Ericaceae) is used in oriental Morocco to treat arterial hypertension. We studied its vasodilator effect and mechanisms of action *in vitro*. The root aqueous extract of *Arbutus* (0.25 mg/ml) produces a relaxation of noradrenaline-precontracted ring preparations of rat aorta with intact endothelium. Relaxation by *Arbutus* did not occur in specimens without endothelium and was inhibited by pretreatment with 100 μ M N^G-methyl-L-arginine (L-NMA), 10 μ M methylene blue or 50 μ M 1H-[1,2,4] oxadiazolo [4,3-a] quinoxaline-1-one (ODQ) but not by 10 μ M atropine. These results suggest that *Arbutus* produces an endothelium-dependent relaxation of the isolated rat aorta which may be mediated mainly by a stimulation of the endothelial nitric oxide synthase by mechanisms other than activation of muscarinic receptors.

Key words : - *Arbutus unedo* – Ericaceae – aorta – vasorelaxation - endothelium – endothelial nitric oxide synthase – NO-sensitive guanylyl cyclase

Running title: *Arbutus* relaxes the isolated rat aorta

INTRODUCTION

Arbutus unedo L. is an Ericaceae species that is known in folk medicine as astringent, diuretic and urinary antiseptic (Grieve, 1967). Phytochemical studies on this plant have led to the isolation of several products (see Karikas & Giannitsaros, 1990). However, very little information is available about the pharmacological actions of *Arbutus*. Our previous survey carried out in oriental Morocco has shown that *Arbutus* is one of the most commonly used plants in this region to treat arterial hypertension (Ziyyat *et al.*, 1997). In the spontaneously hypertensive rat (SHR), oral administration of the root aqueous extract of *Arbutus* has shown vascular, diuretic and natriuretic effects (Ziyyat & Boussairi, 1998). The aim of this work was to examine the vascular effect of *Arbutus* on the isolated rat aorta and to investigate its mechanism of action.

MATERIALS AND METHODS

Plant extract preparation. The extract was prepared by decoction of 40 g of roots in 3 l of distilled water for 2 hours (see Ziyyat & Boussairi, 1998).

Preparation of aorta and experimental device. Male Wistar rats weighing 250-300 g were anaesthetised with sodium pentobarbital (50 mg/kg of body weight, i.p.), and the thoracic aorta was removed carefully in cold physiological salt solution (PSS). After the removal of adhering fatty and connective tissues, an aortic ring of about 2-3 mm in length was suspended between two stainless steel hooks in 10-ml water-jacketed bath containing PSS of the following composition (mM): NaCl 119, KCl 4.7, CaCl₂ 1.6, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25 and glucose 11. The tissue bath solution with pH 7.4 was maintained at 37°C and gassed with 95% O₂ + 5% CO₂. The isometric contraction was recorded via a force-displacement transducer connected to an amplifier and a paper recorder. A tension of 1 g was initially applied to the ring which was

equilibrated in the medium for 30 min. Before each experiment, vasoconstriction was initiated by 1 μ M noradrenaline (N-Adr) in normal PSS and when the steady contraction was reached, 100 μ M carbachol (CCh, a cholinesterase-resistant analogue of acetylcholine) was added to induce endothelium-dependent relaxation. This step was necessary to verify the endothelium integrity. When the relaxation induced by CCh was <50% the experiment was interrupted and another ring was mounted. In denuded aorta, the endothelium was removed mechanically by rubbing the lumen of the artery with plastic tubing. The absence of CCh-induced relaxation was taken as an indicator of successful endothelium denudation.

Experimental protocols

Protocol 1 : Vasorelaxant effect of Arbutus extract on intact aorta. Twenty minutes after the N-Adr/CCh step, contraction of aorta was obtained with 1 μ M N-Adr, after which 0.25 mg/ml of the root aqueous extract of Arbutus (AE) was added in the bath to examine its action. Then, the organ bath was rinsed two times with fresh PSS and the same protocol was repeated after pre-treatment of the ring with 10 μ M atropine (ATR, an inhibitor of muscarinic receptors), 100 μ M N^G-methyl-L-arginine (L-NMA, an inhibitor of NO synthase), 10 μ M methylene blue (MB, a non-selective inhibitor of soluble guanylyl cyclase and/or NO synthase, see e.g. Mayer *et al.*, 1993) or 50 μ M 1H-[1,2,4] oxadiazolo [4,3-a] quinoxaline-1-one (ODQ, a specific inhibitor of the soluble guanylyl cyclase, see Garthwaite *et al.*, 1995). When the action of Arbutus was blocked, 1 μ M sodium nitroprusside (SNP, an NO-donor) was added in the bath to produce an endothelium-independent relaxation.

Protocol 2 : Effect of Arbutus extract on denuded aorta. After contraction of aorta with 1 μ M N-Adr, vascular effects of CCh and Arbutus were tested without rinsing the bath

after each drug. Finally, 1 μ M SNP was added to produce endothelium-independent relaxation.

Chemicals. The following drugs : (-)-Norepinephrine hydrochloride (Noradrenaline), N^G-methyl-L-arginine (L-NMA), Carbamylcholine chloride (Carbachol) were purchased from Sigma Chemical, Atropine from Labosi, Methylene blue from Grraw, Sodium nitroprusside from Farco Chemical and ODQ from Alexis Biochemicals. All compounds were dissolved in saline except ODQ which was dissolved in DMSO.

Statistics. Results are expressed as the means $\pm$ SEM for n separate experiments. Data were analysed by Student's *t*-test and a difference was considered as statistically significant when *p* value was less than 0.05.

RESULTS

Noradrenaline (N-Adr) induced a contraction of intact rat isolated aorta which developed a tension of 0.63 ± 0.03 g (mean $\pm$ s.e.m., n=20) in the presence of 1 μ M of the α -adrenergic agonist (Fig. 1a). Carbachol (CCh, 100 μ M) produced a strong relaxant effect in the presence of N-Adr reducing the α -adrenergic contraction by $75 \pm 3\%$ (Fig. 1a and Table 1). As shown in Fig. 1a, the root aqueous extract of Arbutus (AE, 0.25 mg/ml) produced a similar relaxing effect as CCh on intact N-Adr-precontracted aorta ($77 \pm 4\%$ reduction of contraction, Table 1). However, as shown in Fig. 1b, both vasorelaxant actions of CCh and Arbutus disappeared completely when the endothelium was destroyed (n=6) and the aorta remained contracted. To verify that removal of the endothelium did not damage the vascular muscle underneath, the aorta was challenged with the NO-donor SNP. As shown in Fig. 1b, SNP (1 μ M) still relaxed the endothelium-denuded aorta (on average by $75 \pm 13\%$, n = 4).

The above results indicate that Arbutus produces an endothelium-dependent vasorelaxation similar to that produced by CCh. To examine whether activation of muscarinic receptors was involved in the effect of Arbutus, the vasorelaxant effect of Arbutus was re-examined in the presence of the muscarinic receptor antagonist atropine. As shown in Fig. 2a (summary data in Table 1), atropine (ATR, 10 μ M) did not modify the relaxant effect of Arbutus while it completely antagonized that of CCh. However, the vasorelaxant effect of Arbutus was strongly inhibited in the presence of 100 μ M of the arginine analogue L-NMA (only $11 \pm 2\%$ relaxation, $n=12$, Fig. 2b and Table 1) indicating the participation of NO synthesis in this effect. Moreover, the vasorelaxant effect of Arbutus was completely blunted in the presence of methylene blue (MB, 10 μ M, Fig. 2c) or ODQ (50 μ M, Fig. 2d) suggesting the involvement of cGMP synthesis in this effect. It should be noted that the endothelium-independent vasorelaxant effect of NO was still preserved in the presence of either L-NMA or MB, since 1 μ M SNP still produced a strong relaxation of N-Adr-precontracted aorta in the presence of 100 μ M L-NMA ($84 \pm 5\%$ relaxation, $n=4$) or 10 μ M MB ($87 \pm 3\%$ relaxation, $n=5$). However, the action of SNP was blocked by 50 μ M ODQ (only $3 \pm 2\%$ relaxation, $n=6$) as expected if NO-dependent relaxation is mediated by cGMP. Table 1 summarises the vasorelaxant effects of Arbutus in the absence or presence of the different drugs tested.

DISCUSSION

In the present study, we showed that an aqueous extract prepared from the roots of Arbutus produced a relaxation of precontracted aorta. At 0.25 mg/ml, this effect was comparable to that produced by 100 μ M carbachol. Similar to the effect of CCh (see e.g. Knowles & Moncada, 1992), the vasorelaxant effect of Arbutus disappeared in

denuded aorta, indicating that the integrity of the endothelium was required in order for Arbutus to produce its effect. Moreover, inhibition of NO-synthase by L-NMA or inhibition of soluble guanylyl cyclase by ODQ, which both block the vasorelaxant effect of CCh (see e.g. Lucas *et al.*, 2000), also antagonized the vasorelaxant effect of Arbutus. Although the effect of Arbutus was similar to that of CCh, it was not due to activation of muscarinic receptors since the relaxant effect of Arbutus was unaffected by atropine. We conclude that Arbutus produces an endothelium-dependent relaxation of the aorta which involves activation of endothelial NO-synthase and subsequent cGMP synthesis in the vascular myocytes but does not involve activation of muscarinic receptors.

Interestingly, both the vasorelaxant effects of Arbutus and CCh were blocked by methylene blue which is classically used as an inhibitor of soluble guanylyl cyclase (see e.g. Moncada *et al.*, 1991). However, this compound should be used with caution since it exerts a number of other effects including inhibition of muscarinic receptors (Abi-Gerges *et al.*, 1997) and inhibition of NO-synthase (Mayer *et al.*, 1993). As such, we found here that methylene blue inhibited the effect of CCh on N-Adr-precontracted aorta but left unchanged the relaxant effect of the NO-donor SNP. Therefore, we interpret our results with methylene blue as an effect due to inhibition of NO-synthase rather than inhibition of guanylyl cyclase.

The endothelium-dependent vasodilator effects of Arbutus could be attributed to a number of polyphenolic compounds likely present in the extract. These include tannins and flavonoids which are known for their cardiovascular effects (Fitzpatrick *et al.*, 1993; 1995; Andriambeloson *et al.*, 1998, 1999; Middleton *et al.*, 2000). For example, quercitin (a flavonoid) and tannic acid (a tannin) produce endothelium-dependent

relaxation of rat aortic rings (Fitzpatrick *et al.*, 1993, Flesch *et al.*, 1998) and human coronary arteries and increase cGMP content in both preparations (Flesch *et al.*, 1998). Arbutus roots were shown to contain tannins (Garnier *et al.*, 1961) and aerial parts to contain flavonoids (Dauguet & Foucher, 1982). According to a preliminary phytochemical analysis performed in collaboration with Drs H. Allali and B. Tabti (Laboratory of Organic Chemistry of Tlemcen University , Algeria), both flavonoids and tannins are abundant in the roots of Arbutus (unpublished data). Work is in progress to isolate these compounds, to show their vascular effects *in vitro* and to identify the active principle(s) responsible of the endothelium-dependent vasorelaxation induced by Arbutus.

In conclusion, we showed in this study that Arbutus produces an endothelium-dependent relaxation of aorta which is mainly mediated by a stimulation of endothelial nitric-oxide synthase. We speculate that this effect accounts for the utilisation of Arbutus in folk medicine for the treatment of arterial hypertension.

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REFERENCES

- Abi-Gerges, N., Eschenhagen, T., Hove-Madsen, L., Fischmeister, R., and Méry, P. F. (1997). Methylene blue is a muscarinic antagonist in cardiac myocytes. *Mol. Pharmacol.* **52**, 482-490.
- Andriambeloson, E., Magnier, C., Haan-Archipoff, G., Lobstein, A., Anton, R., Beretz, A., Stoclet, J. C., and Andriantsitohaina, R. (1998). Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. *J. Nutr.* **128** (12), 2324-2333.
- Andriambeloson, E., Stoclet, J. C., and Andriantsitohaina, R. (1999). Mechanism of endothelial nitric oxide-dependent vasorelaxation induced by wine polyphenols in rat thoracic aorta. *J. Cardiovasc. Pharmacol.* **33** (2), 248-254.
- Dauguet, J. C., and Foucher, J. P. (1982). Les flavonoïdes de *Arbutus unedo* L. (Ericacées). *Plant. Med. Phytotherap.* **XVI** **3**, 185-191.
- Fitzpatrick, D. F., Hirschfield, S. L., and Coffey, R. G. (1993). Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am. J. Physiol.* **265**, H774-H778.
- Fitzpatrick, D. F., Hirschfield, S. L., Ricci, T., Jantzen, P., and Coffey, R. G. (1995). Endothelium-dependent vasorelaxation caused by various plant extracts. *J. Cardiovasc. Res.* **26**, 90-95.
- Flesch, M., Schwarz, A., and Bohm, M. (1998). Effects of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arteries. *Am. J. Physiol.* **275**, H1183-H1190.
- Garnier, G., Bezanger-Beauquesne, L., and Debraux, G. (1961). Ressources Médicinales de la Flore Française, Vol. **2**, pp. 962-964, Vigot Frères, Paris.
- Garthwaite, J., Southam, E., Boulton, C. L., Nielson, E. B., Schmidt, K., and Mayer, B. (1995). Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1-H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. *Mol. Pharmacol.* **48**, 184-188.

- Grieve, M. (1967). A Modern Herbal. Vol. **1**, p. 53, Hafner Publishing Co., New York and London.
- Karikas, G. A., and Giannitsaros, A. (1990). Glucosides phénoliques des feuilles d'*Arbutus unedo*. *Plant. Med. Phytother.* **XXIV** **1**, 27-30.
- Knowles, R. G., and Moncada, S. (1992). Nitric Oxide as a Signal in Blood Vessels. *Trends Biochem. Sci.* **17**, 399-402.
- Lucas, K. A., Pitari, G. M., Kazerounian, S., RuizStewart, I., Park, J., Schulz, S., Chepenik, K. P., and Waldman, S. A. (2000). Guanylyl cyclases and signaling by cyclic GMP. *Pharmacol. Rev.* **52**, 375-413.
- Mayer, B., Brunner, F., and Schmidt, K. (1993) Inhibition of nitric oxide synthesis by methylene blue. *Biochem. Pharmacol.* **45**, 367-374.
- Middleton, E. Jr, Kandaswami, C., and Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implication for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **52**, 637-751.
- Moncada, S., Palmer, R. M. J., and Higgs, E. A. (1991). Nitric Oxide - Physiology, Pathophysiology, and Pharmacology. *Pharmacol. Rev.* **43**, 109-142.
- Ziyyat, A. Legssyer, A., Mekhfi, H., Dassouli, A., Serhrouchni, M., and Benjelloun, W. (1997). Phytotherapy of hypertension and diabetes in oriental Morocco. *J. Ethnopharmacol.* **58**, 45-54.
- Ziyyat, A., and Boussairi, E. (1998). Cardiovascular effects of *Arbutus unedo* L. in spontaneously hypertensive rats. *Phytother. Res.* **12**, 110-113.

Table 1. Mean values of the vasorelaxant effect (expressed as percent of relaxation on intact noradrenaline-precontracted aorta) of carbachol (CCh) and aqueous extract (AE) of *Arbutus unedo* in absence and in presence of atropine, L-NMA, methylene blue (MB) or ODQ.

	CCh 100 μ M	AE 0.25 g/l				
		AE alone	+ Atropine 10 μ M	+ L-NMA 100 μ M	+ MB 10 μ M	+ ODQ 50 μ M
Mean	75 $\pm$ 3 (NS)	77 $\pm$ 4	86 $\pm$ 4 (NS)	11 $\pm$ 2 ^a	0.4 $\pm$ 0.4 ^a	0 ^a
n	20	20	6	12	4	6

Values are mean $\pm$ SEM. ^a $p < 0.001$ vs AE alone ; NS : not significant compared to AE alone.

Legend of figures

Figure 1

Typical tracings showing the effect of carbachol (CCh, 100 μ M) and root aqueous extract of *Arbutus* (AE, 0.25 mg/ml) on noradrenaline (N-Adr, 1 μ M) pre-contracted intact (a) and denuded rat aorta (b). Sodium nitroprusside (SNP, 1 μ M) was added to produce an endothelium-independent relaxation. W indicates the moment when all drugs were washed out.

Figure 2

Original tracings showing the vascular effect of carbachol (CCh, 100 μ M) and aqueous extract of *Arbutus* (AE, 0.25 mg/ml) on noradrenaline (N-Adr, 1 μ M) pre-contracted intact rat aorta in presence of : (a) atropine (ATR, 10 μ M); (b) N^G-methyl-L-arginine (L-NMA, 100 μ M); (c) methylene blue (MB, 10 μ M); or (d) ODQ 50 μ M. Endothelium-independent relaxation was obtained with sodium nitroprusside (SNP, 1 μ M). W indicates the moment when all drugs were washed out.