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Delphinidin-3-rutinoside relaxes the bovine ciliary smooth muscle through activation of ET_B receptor and NO/cGMP pathway

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Abstract

Delphinidin-3-rutinoside (D3R) is the major anthocyanin component in blackcurrant (*Ribes nigrum* L.) fruits. We investigated the relaxation mechanism of D3R in bovine ciliary smooth muscle (CM). D3R at a concentration of 10^{-5} M produced a sustained and progressive relaxation during the contraction induced by endothelin (ET)-1 in the bovine CM specimens. After the pre-treatment with D3R, the anthocyanin exerted an inhibitory effect on the ET-1-induced contraction with a concomitant increase in cyclic GMP production and decreased phosphorylation ratio of myosin light chain (RLC). The inhibitory effect of D3R was significantly attenuated in the presence of either N^G-nitro-L-arginine (NOARG) as a nitric oxide synthase (NOS) inhibitor, carboxy-PTIO as a NO scavenger, ODQ as an inhibitor of guanylyl cyclase, or BQ788 as a selective ET_B receptor antagonist. The atteuation with NOARG was reversed by the addition of excess L-arginine. However, iberiotoxin as a Ca^{2+}-activated K^+ channel inhibitor, propranolol as a β-adrenoceptor antagonist, and indomethacin as a cyclooxygenase inhibitor failed to modify the inhibitory effect of D3R. Scatchard plot analysis revealed that the [^{125}I]-ET-1 binding site constituted a single population with Kd of $54{\cdot}5 \pm 4{\cdot}6$ nM and maximum binding site (B_{max}) of $168{\cdot}4 \pm 25{\cdot}4$ fmol/mg protein in the ciliary epithelium (CE), and Kd of $141{\cdot}7 \pm 18{\cdot}0$ nM and B_{max} of $357{\cdot}7 \pm 35{\cdot}8$ fmol/mg protein in CM. [^{125}I]-ET-1 binding was completely displaced by BQ788 with K_i values of $56{\cdot}7 \pm 10{\cdot}8$ pM in CE and $93{\cdot}4 \pm 23{\cdot}3$ pM in CM. Meanwhile, partial displacement (approximately 40%) was observed by BQ123 as a selective ET_A receptor antagonist in both preparations. ET_B receptor was predominant subtype in CE and CM, whereas kinetics of the binding was different in two preparations. These results suggest that D3R possibly stimulates ET_B receptors to produce/release NO, and results in an inhibition of myosin RLC phosphorylation and/or acceleration of dephosphorylation, thereby causing relaxation and producing an inhibitory effect on the ET-1-induced contraction in the bovine CM.

Keywords: ciliary smooth muscle; relaxation; NO; endothelin receptor; delphinidin-3-rutinoside; anthocyanin; blackcurrant

1. Introduction

Myopia may be due to axial elongation of the globe, inappropriate refractive power of the eye, or a combination of both. One theory for the development of refractive myopia is that the ciliary muscle becomes spastic as a result of excessive contraction during close up work, leading to spasmodic refractive power of the lens. As a consequence, the myopic ciliary muscle cannot relax sufficiently to allow the lens to focus on distant images (Tokoro, 1998).

Contraction of the ciliary muscle is mediated by the excitation of several types of receptors (Kamikawatoko et al., 1995; Azuma et al., 1997), while relaxation is governed by two independent mechanisms, one is cyclic AMP-dependent that includes β-adrenergic and prostaglandin receptor-mediated responses, and the other is cyclic AMP-independent that involves an NO-related mechanism (Goh et al., 1995). Kamikawatoko et al. (1995) reported that relaxation of bovine ciliary muscle strips was regulated by NO release similar to vascular smooth muscle.

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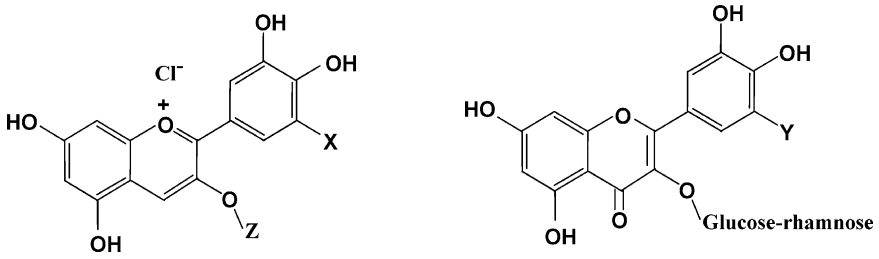

Fig. 1. Four forms of anthocyanins are found in blackcurrant, delphinidin-3-rutinoside (X=OH, Z=glucose-rhamnose), delphinidin-3-glucoside (X=OH, Z=glucose), cyanidin-3-glucoside (X=H, Z=glucose) and cyanidin-3-rutinoside (X=H, Z=glucose-rhamnose). In addition, the berries contain two forms of flavonoids, myricetin-3-rutinoside (X=OH) and quercetin-3-rutinoside (X=H).

The cyclic AMP-independent, non-autonomic mechanism of ciliary muscle relaxation has been extensively studied in the search for drug therapies to treat both myopia and glaucoma (Beauregard et al., 2001).

Endothelins are a group of peptides containing 21 amino acids and were first described by Yanagisawa et al. (1988). ET-1 mediates contraction in various smooth muscle systems such as the vasculature of ciliary muscle. The ET_A receptor, which is localized on vascular smooth muscle cells, mediates potent vasoconstrictor actions (Masaki et al., 1991). The ET_B receptor is localized on vascular endothelial cells and thought to mediate vasodilatation through the release of nitric oxide and prostaglandins (Inoue et al., 1989). In anterior eye, the presence of ET system has been investigated. In bovine ciliary muscle, Kamikawatoko et al. (1995) demonstrated that ET-1 caused contraction at high concentrations (10^{-9} and 10^{-8} M) and relaxation at low concentrations (10^{-11} and 10^{-10} M). They suggested that the contraction was mediated through excitation of ET_A, whereas the relaxation was mediated by ET_B. In rat ciliary body, Ripodas et al. (1998) reported the existence of ET_A and ET_B receptor subtypes in a ratio of 35:65 and their localization on the CE. Fernández-Durango et al. (2003) also reported the mRNA expression pattern of ET-1, ET_A and ET_B receptor genes in the CM and ciliary processes of human eyes by the in situ hybridization and immunohistochemistry. These reports suggested ET-1, ET_A and ET_B receptors play an important role for the visual function.

Dietary anthocyanins have attracted considerable interest due to their health-promoting benefits, such as reducing the risk of coronary heart disease and preventing several chronic diseases (Renaud and de Logeril, 1992). There is also evidence that a mixture of anthocyanins from bilberry (*Vaccinium myrtillus* L.) have ophthalmic activity since the extract promoted recovery of visual acuity in cases of pseudomyopia in primary school students (Kajimoto et al., 2000). Although bilberry is widely available as a nutritional supplement in the United States and Japan, its pharmacological properties have not been extensively studied and there are only a limited number of references on its effects on visual function.

Black currant (*Ribes nigrum* L.) berries and juice are also rich in anthocyanins, and are consumed in many countries of the world. We have reported previously the development of a powdered concentrate of black currant anthocyanins (BCA) from a commercial source (Matsumoto et al., 2001a). The composition of this concentrate is summarized in Fig. 1 and consists of four anthocyanin and two flavonoid components that include delphinidin-3-rutinoside (D3R), delphinidin-3-glucoside (D3G), cyanidin-3-rutinoside (C3R), cyanidin-3-glucoside (C3G), myricetin-3-rutinoside (M3R) and quercetin-3-rutinoside (Q3R), respectively. The four anthocyanins have been successfully isolated and purified (Matsumoto et al., 2001a) and we have demonstrated these compounds are absorbed through the gastrointestinal tracts and can be detected in the blood as unmetabolized forms in both humans and rats (Matsumoto et al., 2001b). In a series of studies, we recently described the pharmacological properties of these anthocyanins (Nakamura et al., 2002; Matsumoto et al., 2002, 2003) and showed that oral intake of BCA prevented myopic refractory shift caused by working on visual display terminals (VDT) (Nakaishi et al., 2002). This led us to assume that the beneficial effect of anthocyanins results from the relaxation of CM that had undergone spasmodic contractions.

In the present study, we found that D3R showed both relaxation activity during the contraction caused by ET-1 and inhibitory effect on the ET-1-induced contraction in bovine CM. We attempted to clarify the signal-transduction pathways affected by D3R that governed the relaxation mechanism. This study represents the first scientific investigation on the pharmacological effects of dietary anthocyanins on CM and incorporated a comparison of the relationship between structure and relaxation activity in CM of 4 polyphenolic rutinoside components purified from blackcurrant, D3R, C3R, M3R and Q3R.

2. Materials and method

2.1. Chemicals and solutions

Carbamylcholine chloride (carbachol), 3-isobutyl-1-methylxanthine (IBMX) as a non-selective inhibitor of phosphodiesterases, L-arginine as nitric oxide synthase substrate, N^G-nitro-L-arginine (NOARG) as an inhibitor of nitric oxide synthase, indomethacin as a cyclooxygenase