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Protective Effect of DHQ-11 against Hypoxia-induced Vasorelaxation

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Abstract

Hypoxia, or the lack of oxygen, has multiple impacts on the vascular system. The major molecular sensors for hypoxia at the cellular level are hypoxia inducible factor and heme oxygenase. Hypoxia also acts on the vasculature directly conveying its damaging effects through disruption of the control of vascular tone, particularly in the coronary circulation, enhancement of inflammatory responses and activation of coagulation pathways. These effects could be particularly detrimental under pathological conditions such as obstructive sleep apnea and other breathing disorders. Introduction: The effect of conjugate 2-(3,4-Dihydroxyphenyl)-6-{[1-(2'-bromo-3',4'-dimethoxyphenyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl]methyl}-3,5,7-trihydroxychroman-4-one (DHQ-11) on hypoxia-induced vasorelaxation was investigated in rat aortic rings using standard organ bath techniques. Materials and methods: Hypoxia was stimulated by a superfusion of aortic rings with a glucose-free Krebs solution bubbled with 95 % N_2/5 % CO_2. The effect of conjugate DHQ-11 was assessed after a 60- min period of hypoxia on aortic rings precontracted with 50 mM KCl or 1μM phenylephrine (PE). The conjugate DHQ-11 significantly attenuated hypoxia-induced vasorelaxation in the endothelium-intact aortic rings precontracted with KCl or PE in a concentration-dependent manner. Results and discussion: This effect of conjugate DHQ-11 was more potent in aortic rings precontracted with PE than those with KCl where it maximally reduced hypoxia-induced vasorelaxation from 44.7 ± 3.7 to 5.4 ± 3.7 and 33.9 ± 3.4 to 10.8 ± 4.2 %, respectively. The removal of the endothelium attenuated the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation. Similarly, pretreatment of endothelium-intact aortic rings with L-NAME and methylene blue also attenuated the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation. Furthermore, the blockade of the ATP-sensitive K_{ATP} channel with glibenclamide and the calcium-activated large conductance BK_{Ca} channel with TEA also significantly attenuated the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation. Collectively, these results indicated that conjugate DHQ-11 attenuated the hypoxia-induced vasorelaxation suggesting that it alleviated the oxidative damage of vasculature. Conclusions: This effect of conjugate DHQ-11 possible is mediated through several mechanisms including the blockage of the extracellular Ca^{2+} entry via the voltage-dependent and receptor-operative Ca^{2+} channels, as well as inhibition of sarcoplasmic reticulum Ca^{2+} release via the inositol triphosphate pathway. In addition,

endothelium and NO/sGC/cGMP/PKG pathway, as well as K_{ATP} and BK_{Ca} channels most likely participate in protection by conjugate DHQ-11 against hypoxia-induced vasorelaxation.

Keywords: Smooth muscle, Endothelium, Nitric oxide, Ca-channels, K^+ channels, Hypoxia

Introduction

Cardiovascular diseases (CVDs), a group of disorders of the heart and blood vessels that are the leading cause of death worldwide, remain the major global health problem [1,2]. A growing body of evidence demonstrates that hypoxia is one of the common features in the pathophysiology of a variety of cardiovascular disorders including arterial hypertension [3,4]. The pathogenesis of arterial hypertension involves multiple pathological factors among which oxidative stress arising from the excessive production of reactive oxygen species (ROS) plays a critical role [5,6]. The increased generation of reactive oxygen species (ROS) under hypoxia-ischemia led to disruption of Ca^{2+} homeostasis which is a major determinant of smooth muscle contractility [7]. The critical role in maintaining Ca^{2+} homeostasis in smooth muscle cells plays Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels (VDCCs) and Ca^{2+} released from the sarcoplasmic reticulum (SR) via inositol 1,4,5-phosphate receptor (IP_3R) [8,9]. There is evidence that these Ca^{2+} handling proteins of smooth muscle cells are significantly altered under hypoxia, leading to the impairment of vascular contractile performance [10]. Moreover, it has also been reported that an important role in hypoxia-induced vasorelaxation plays activation of K^+ channels that leads to hyperpolarization and subsequent inhibition of VDCCs, resulting in the decreased level of $[Ca^{2+}]_i$ and vasorelaxation [11,12]. Hypoxia affects Ca^{2+} homeostasis in smooth muscle cells also through impairment of vascular endothelium function which plays an important role in the regulation of vascular contractility [13]. Endothelial dysfunction is characterized by reduced vasorelaxation response to vasoactive substances and loss of nitric oxide (NO)-bioavailability due to reduced endothelial NO synthase (eNOS) activity and accelerated degradation of NO [14]. NO plays a key role in the mechanisms of endothelium-mediated vasorelaxation that involve activation of sGC/cGMP/PKG pathway leading to inhibition of VDCCs and facilitation of Ca^{2+} uptake by Ca^{2+}-ATPase of SR resulting in the decrease of intracellular level of Ca^{2+} ($[Ca^{2+}]_i$) [15].

Recently we have found that DHQ-11, a conjugate of flavonoid dihydroquercetin with isoquinoline alkaloid 1-aryl-6,7-dimethoxy-1,2.3,4-tetrahydroisoquinoline, exerts a potent vasorelaxant effect mediated through endothelium-independent and endothelium-dependent mechanisms. The endothelium-independent mechanism involves the inhibition of Ca^{2+} influx through direct blockage of VDCCs and indirectly by activation of K_{ATP} and BK_{Ca} channels whereas the endothelium-dependent mechanism is mainly mediated by the activation eNOS activity and NO-sGC-cGMP pathway. Therefore, the present study aims to explore the potential protective effects of conjugate DHQ-1 against hypoxia-induced vasorelaxation in rat aortic rings.

Materials and methods

Tissue preparation

All experimental protocols and conditions for preoperative care were approved by the animal use committee of our institution. Adult male Wistar rats weighing 200 - 250 g were anesthetized with sodium pentobarbital and their thorax was opened and the thoracic aorta was quickly removed and immediately placed in Krebs solution contained (in mmol/L); 118 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 11 mM glucose. All the dissection procedures were performed

with extreme care to protect the endothelium from inadvertent damage. The aorta was cleaned of adipose and connective tissue and cut into rings (2 - 3 mm long).

Aortic-ring contraction studies

The aortic rings were mounted using 2 stainless hooks with 1 fixed to the bottom of the organ bath (Radnoti Glass, NSW, AUS) and the other connected to a force transducer. The organ bath was superfused with Krebs solution, bubbled with a 95 % O_2/5 % CO_2 gas mixture, and maintained at 37 C. The aortic rings were equilibrated in Krebs solution under the tension of 1 g, for 60 min during which period the Krebs solution was replaced at least twice. Aortic ring contraction was recorded, isometrically using a force transducer (FT-03; Grass Instrument Company, USA) coupled to a computer-based data acquisition system (PowerLab, ADInstruments) connected to a chart recorder Endim 621- 02 (Germany).

Experimental protocols

The effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation was assessed using aortic rings incubated in a glucose-free Krebs solution with a constant supply of 95 % N_2/5 % CO_2. After a 60 min period of hypoxia which is commonly used in these studies, aortic rings were precontracted with 50 mM KCl or 1 μM phenylephrine (PE). The decrease in force in response to hypoxia was characterized in terms of the maximum hypoxic vasorelaxation (at ~60 min), expressed as a percentage of the contraction force induced by 50 mM KCl or 1μM PE immediately preceding aeration with 95 % N_2 / 5 % CO_2.

To examine the involvement of the VDCCs in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation its effects in presence of nifedipine, a specific inhibitor of these channels, were studied.

To investigate the involvement of calcium release from the SR in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation its effects on PE-induced contraction of endothelium-denuded aortic rings in Ca^{2+}-free buffer containing EGTA (1 mM) was studied.

To evaluate the contribution of the K^+ channels in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation, its effects in presence of the glibenclamide, a specific inhibitor of ATP-sensitive K^+ channel (K_{ATP}), tetraethylammonium chloride. (TEA), a nonspecific inhibitor of the calcium-activated large conductance K^+ channel (BK_{Ca}) and $BaCl_2$, a specific inhibitor of the inward rectifying K^+ channels (K_{IR}), were studied. These studies were performed on endothelium-intact aortic rings precontracted with 30 mM KCl to depolarise the membrane and activate K^+ channels since in this condition effect of drugs on K^+ channels is more potent [16].

To assess the role of the endothelium in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation the endothelium was removed from ring specimens by rubbing the intimal surface with a cotton ball and the absence of ACh-induced relaxation was taken as an indicator of successful denudation. To further clarify the role of the endothelium in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation its effects on PE-induced contraction of endothelium-intact aortic rings preincubated with L- NAME (nitro-L-arginine methyl ester, a NO synthase inhibitor), methylene blue (a guanylyl cyclase inhibitor) and indomethacin (a cyclooxygenase inhibitor), were studied.

Chemicals

All chemicals were of analytical grade commercially available. Phenylephrine, nifedipine, EGTA, acetylcholine, L-NAME, methylene blue, indomethacin, glibenclamide, TEA, and $BaCl_2$ were obtained from Sigma Ltd Co., (St. Louis, MO, USA). Conjugate DHQ-11 was synthesized at the Institute of Chemistry of Plant Compounds of Uzbek Academy of Sciences and was kindly provided by V.I. Vinogradova.

Statistics

Throughout this article, all data are represented as the mean standard error of the mean (s.e.m.) of *n* observations. Statistical analysis was performed using an unpaired Student's *t*-test. The EC_{50} and IC_{50} values, the concentration of drugs causing a 50 ℃ contraction or relaxation of the maximal response (E_{Max}), were obtained from the concentration-response curve and calculated using the sigmoidal curve fitting routine in Origin 6.0 (Microcal, Northampton, MA, U.S.A.). The differences between control and experimental values were considered significant at $p < 0.05$.

Results and discussion

Effect of conjugate DHQ-11 on the hypoxia-induced vasorelaxation

In control experiments, exposure of endothelium-intact aortic rings to Krebs solution gassed with 95 % N_2/5 % CO_2 for 60 min significantly reduced contractile response to KCl or PE. As shown in **Figure 1**, the response of aortic rings to KCl (50 mM) and PE (1μM) was decreased by 33.9 ± 3.4 and 44.7 ± 3.7 % , respectively, of the control response in normoxia. These results show that stimulated hypoxia- induced vasorelaxation which was more potent in aortic rings precontracted with PE than KCl indicating that the contractile response to PE is more sensitive to hypoxia than is that to KCl. It was found that the pretreatment of the endothelium-intact aortic rings with conjugate DHQ-11 significantly inhibited the vasorelaxation induced by hypoxia in aortic rings precontracted both with KCl and PE (**Figure 1**). As demonstrated in **Figure 1**, the conjugate DHQ-11 maximally inhibited hypoxia-induced vasorelaxation in the aortic rings precontracted with KCl (50 mM) from 33.9 ± 3.4 to 10.8 ± 4.2 % at the concentration of 14.8 μM. Similarly, on the aortic rings precontracted with PE (1 μM) the conjugate DHQ-11 maximally inhibited hypoxia-induced vasorelaxation from 44.7 ± 3.7 to 5.4 ± 3.7 % at the concentration of 23.7 μM (**Figure 1(B)**).

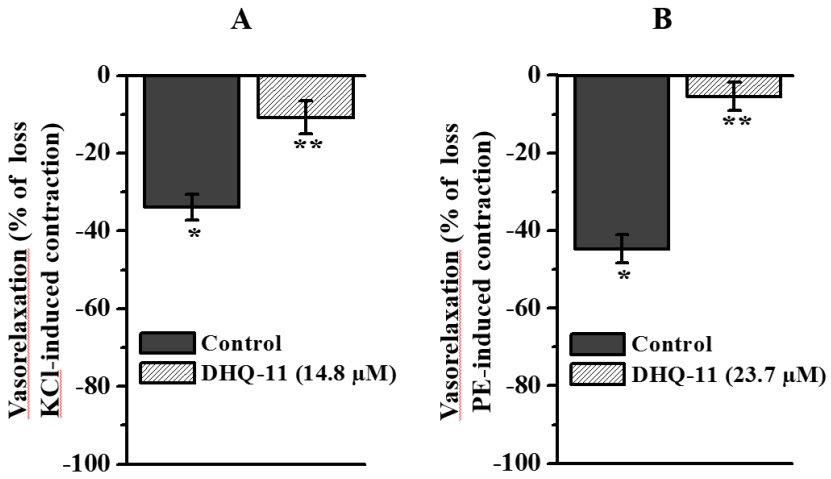

Figure 1 Effect of the conjugate DHQ-11 on the hypoxia-induced vasorelaxation in rat aortic rings precontracted with (A) KCl and (B) phenylephrine.

The endothelium-intact aortic rings were preincubated for 60 min in Krebs solution gassed with 95 % N_2/5 % CO_2 and precontracted with 50 mM KCl and 1 μM phenylephrine (PE). Vasorelaxation was expressed as the percentage of inhibition of maximal contraction induced by KCl and PE. Data are presented as mean ± SEM ($n = 7$). *$p < 0.05$, **$p < 0.01$, vs control.

These results show that the effect of conjugate DHQ-11 on the vasorelaxation induced by hypoxia is more potent in aortic rings precontracted with PE than with KCl. It was reported that vasorelaxation induced by hypoxia is mainly due to a reduction in $[Ca^{2+}]_i$ in the smooth muscle cells resulting from the inhibition of Ca^{2+} influx through VDCCs and calcium release from the SR via IP_3Rs [17]. To examine the involvement of the VDCCs in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation its effects in presence of nifedipine, a specific inhibitor of these channels, were studied. In these studies was found that in aortic rings preincubated with nifedipine (0.01 μM) and precontracted with KCl the inhibitory effect of conjugate DHQ-11 on the vasorelaxation induced by hypoxia decreased from 23.1 ± 4.2 to 6.7 ± 3.3 % (**Figure 2**).

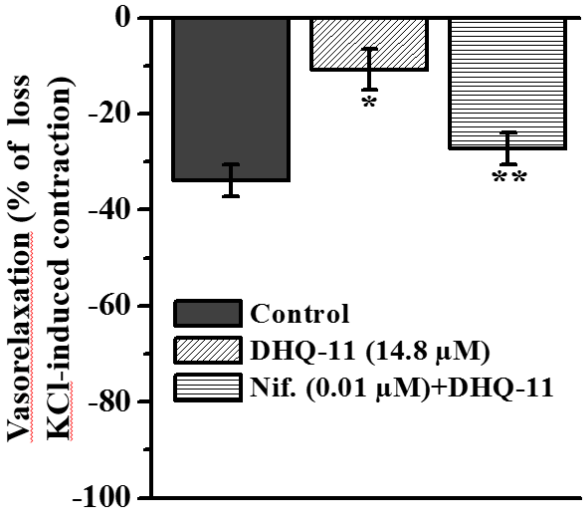

Figure 2 Influence of nifedipine (nif) on the effect of conjugate DHQ-11 on the hypoxia-induced vasorelaxation in endothelium-intact rat aortic rings precontracted with KCl

The endothelium-intact aortic rings were preincubated for 60 min in Krebs solution gassed with 95 % N_2/5 % CO_2 and precontracted with 50 mM KCl. Vasorelaxation was expressed as the percentage of inhibition of maximal contraction induced by KCl. Data are presented as mean ± SEM (n = 5). *p < 0.05, **p < 0.01, vs control.

This result suggests that L-type VDCCs are involved in the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation. To test the possible involvement of the calcium release from the SR in the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation its effect on endothelium-denuded aortic rings precontracted with PE in the Ca^{2+}-free buffer was studied. In these experimental conditions PE-induced aortic rings contraction which is mainly due to the release of Ca^{2+} from SR through IP_3Rs. In this study, we found that the conjugate DHQ-11 significantly inhibited the vasorelaxation induced by hypoxia in aortic rings precontracted with PE in Ca^{2+}-free buffer. Results present in **Figure 3** show that the effect of conjugate DHQ-11 (23.7 μM) on the vasorelaxation induced by hypoxia in these conditions significantly decreased from 57.2 ± 3.5 to 41.7 ± 3.7 %.

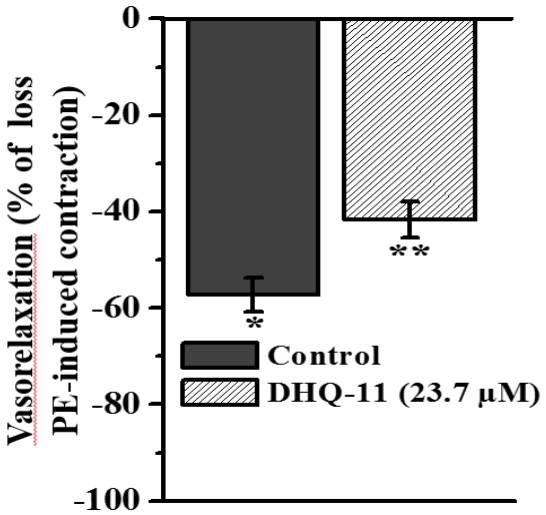

Figure 3 Effect of the conjugate DHQ-11 on the hypoxia-induced vasorelaxation in endothelium-intact rat aortic rings precontracted with phenylephrine in Ca^{2+} - free Krebs solution.

The endothelium-intact aortic rings were preincubated for 60 min in Ca^{2+} - free Krebs solution gassed with 95 % N_2/5 % CO_2 and precontracted with 1µM phenylephrine (PE). Vasorelaxation was expressed as the percentage of inhibition of maximal contraction induced by PE in Ca^{2+}- free Krebs solution. Data are presented as mean ± SEM (n = 5). *p < 0.05, **p < 0.01, as compared with the control.

These results indicated that the modulation of calcium release from the SR also may be involved in the effect of conjugate DHQ-11 on the vasorelaxation induced by hypoxia.

The involvement of the K^+ channels in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation

It was demonstrated that voltage-gated (K_V7) and ATP-sensitive potassium (K_{ATP}) channels that control the smooth muscle contractility and vascular tone play an important role in hypoxia-induced vasorelaxation [11,12]. To examine the possible involvement of the K^+ channels in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation its effects in presence of glibenclamide, a specific inhibitor of K_{ATP} channel, TEA a nonspecific inhibitor of BK_{Ca} channel, and, $BaCl_2$ a potent inhibitor of K_{IR} channel, were studied. These studies were performed in the aortic rings precontracted with 30 mM KCl since in this condition effect of drugs on K^+ channels is more potent [18,19]. As shown in **Figure 4** in the intact aortic ring preincubated with glibenclamide (50 µM) and precontracted with KCl (30 mM) the inhibitory effect of conjugate DHQ-11(14.8 µM) on hypoxia-induced vasorelaxation decreased from 36.9 ± 4.1 to 14.8 ± 3.4 %. Similarly, in the presence of TEA (1 mM) the effect of conjugate DHQ-11 (14.8 µM) on hypoxia-induced vasorelaxation decreased from 36.9 ± 4.1 to 20.0 ± 3.6 % (**Figure 4**). By contrast, in the presence of $BaCl_2$ (100 µM), the effect of conjugate DHQ-11 (14.8 µM) on hypoxia-induced vasorelaxation reduced not significantly (**Figure 4**).

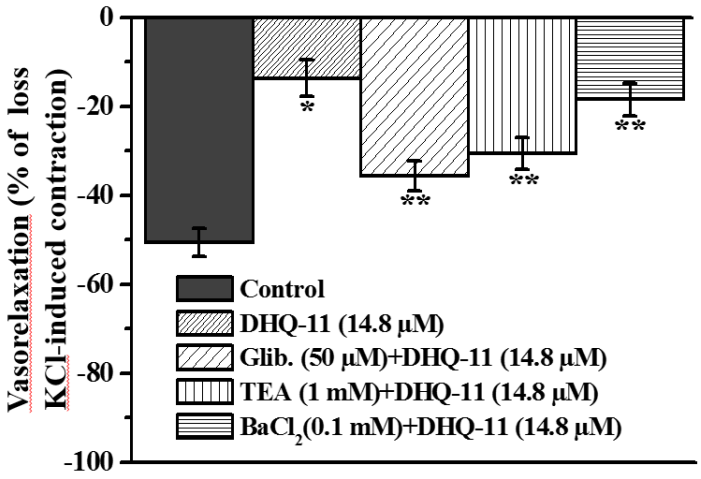

Figure 4 Effect of the conjugate DHQ-11 on hypoxia-induced vasorelaxation in intact aortic rings in the presence of K^+channel blockers.

Endothelium-intact aortic rings were preincubated for 60 min in Krebs solution gassed with 95 % N_2/5 % CO_2 in the presence of 10 μM glibenclamide (Glib), 1 mM TEA, and 30 μM $BaCl_2$ and precontracted with 30 mM KCl. Vasorelaxation was expressed as the percentage of inhibition of maximal contraction induced by KCl. Data are presented as mean ± SEM (n = 6). *p < 0.05, **p < 0.01, vs control.

These results demonstrate that preincubation of aortic rings with glibenclamide and TEA significantly abolished the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation suggesting that K_{ATP} and BK_{Ca} channels could be involved in this effect.

The involvement of endothelium in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation

It was shown that a critical role in hypoxia-induced vasorelaxation plays changes in the mechanisms mediating endothelium-dependent contraction and relaxation which control vascular tone [20]. To test the involvement of endothelium in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation its effect was evaluated using the aortic rings with removed endothelium. After removal of the endothelium by rubbing the intimal surface with a cotton ball the vasorelaxation induced by hypoxia in aortic rings precontracted with KCl (50 mM) and PE (1μM) increased from 33.9 ± 3.4 to 41.9 ± 4.8 % and from 44.7 ± 3.7 to 55.8 ± 3.5 % , respectively (**Figure 5**). These results show that removal of the endothelium enhances the vasorelaxation induced by hypoxia. In the endothelium-denuded aortic rings preincubated with the conjugate DHQ-11 (14.8 μM) and precontracted with KCl (50 mM) hypoxia-induced vasorelaxation reduced from 41.9 ± 4.8 to 30.6 ± 3.9 % (**Figure 5(A)**). In similar experiments in the endothelium-denuded aortic rings precontracted with PE (1 μM) the conjugate DHQ-11 (23.7 μM) reduced hypoxia-induced vasorelaxation from 55.8 ± 3.5 to 27.9 ± 2.8 % (**Figure 5(B)**). The results indicate that removal of endothelium more markedly attenuated the effect of the conjugate DHQ-11 on vasorelaxation induced by hypoxia in the aortic rings precontracted with PE. To further clarify the involvement of the endothelium in the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation its effect on endothelium-intact aortic rings preincubated with inhibitors of NOS - L-NAME, guanylate cyclase-methylene blue, and cyclooxygenase-indomethacin, were studied.

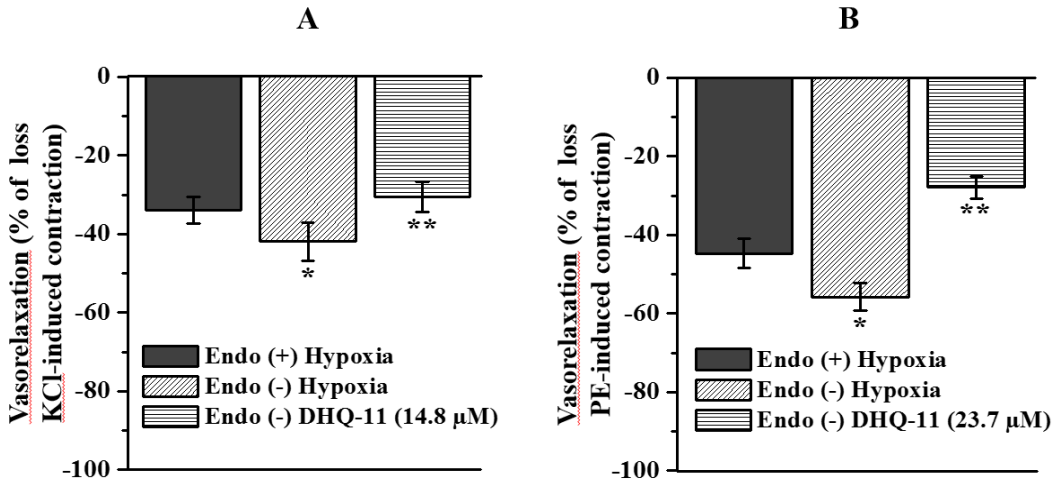

Figure 5 Effect of the conjugate DHQ-11 on hypoxia-induced vasorelaxation in the endothelium-intact and endothelium denuded aortic rings precontracted with (A) KCl and (B) phenylephrine.

The aortic rings with intact (E+) and removed endothelium (E-) were preincubated for 60 min in Krebs solution gassed with 95 % N_2/5 % CO_2 and precontracted with 50 mM KCl and 1µM phenylephrine (PE). Vasorelaxation was expressed as the percentage of inhibition of maximal contraction induced by KCl and PE. Data are presented as mean ± SEM (n = 5). *p < 0.05, **p < 0.01, vs control.

Table 1 Influence of L-NAME, methylene blue, and indomethacin on the effect of the conjugate DHQ-11 on hypoxia-induced vasorelaxation in endothelium-intact aortic rings precontracted with KCl and PE.

Experimental Group	Vasorelaxation %			
	Precontracted by KCl (50 mM)		Precontracted by PE (1 µM)	
	Hypoxia	Hypoxia DHQ-11	Hypoxia	Hypoxia DHQ-11
Control	33.9 ± 3.4*	10.8 ± 4.2**	44.7 ± 3.7*	5.4 ± 3.7**
L-NAME (100 µM)	39.6 ± 4.1**	26.4 ± 3.8*	52.5 ± 3.9*	24.7 ± 4.2**
Methylene blue (10 µM)	38.2 ± 3.7*	23.3 ± 4.2**	50.7 ± 4.4*	20.9 ± 4**
Indomethacin (10 µM)	34.4 ± 3.9**	13.6 ± 3.5**	47.3 ± 3.2*	8.6 ± 4.1**

The aortic rings with intact endothelium preincubated for 60 min in Krebs solution gassed with 95 % N_2/5 % CO_2 in the presence of L-NAME, methylene blue and indomethacine and precontracted with 50 mM KCl and 1µM phenylephrine (PE). Vasorelaxation was expressed as the percentage of inhibition of maximal contraction induced by KCl and PE. Data are presented as mean ± SEM (n = 5). *p < 0.05, **p < 0.01, vs control.

The results presented in **Table 1** show that in the endothelium-intact aortic ring preincubated with L-NAME (100 μM) and precontracted with KCl (50 mM) and PE (1 μM) the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation reduced from 23.1 ± 4.2 to 7.5 ± 3.8 % and from 39.3 ± 3.7 to 20.0 ± 4.2 %, respectively. In a similar experiment with methylene blue (10 μM) in the endothelium-intact aortic ring precontracted with KCl (50 mM) and PE (1 μM) the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation was reduced from 23.1 ± 4.2 to 10.6 ± 4.2 % and from 39.3 ± 3.7 to 23.8 ± 4.0 %, respectively. In the presence of indomethacin (10 μM) the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation was reduced from 23.1 ± 4.2 to 20.3 ± 3.5 % and from 39.3 ± 3.7 to 36.1 ± 4.1 %, respectively. These results demonstrate that L-NAME and methylene blue significantly reduced the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation suggesting that this effect of conjugate DHQ-11 is likely associated with modulation of NO/sGC/cGMP/PKG pathway.

Discussion

Recently we have shown that DHQ-11, a conjugate of flavonoid dihydroquercetin with isoquinoline alkaloid 1-aryl-6,7-dimethoxy-1,2.3,4-tetrahydroisoquinoline, exerts a potent vasorelaxant effect. In this study, we have found that conjugate DHQ-11 also significantly inhibited the vasorelaxation induced by hypoxia in aortic rings precontracted both with KCl and PE. This inhibitory effect of conjugate DHQ-11 on the vasorelaxation induced by hypoxia was more potent in aortic rings precontracted with PE than with KCl. It was reported that vasorelaxation induced by hypoxia is mainly due to a reduction in $[Ca^{2+}]_i$ in the smooth muscle cells resulting from the inhibition of Ca^{2+} release from the SR and Ca^{2+} influx through VDCCs [21]. At the same time was shown that the contractile response to PE which is mainly mediated by Ca^{2+} released from the SR is more sensitive to hypoxia than is that to KCl. This is explained by the fact that Ca^{2+} release from the SR via IP_3Rs requires much energy from ATP and therefore its activity is significantly altered in hypoxia. In the present study, we have found that the conjugate DHQ-11 also markedly inhibited the vasorelaxation induced by hypoxia in aortic rings precontracted with PE in Ca^{2+}-free buffer. These results indicated that the inhibitory effect of conjugate DHQ-11 on the vasorelaxation induced by hypoxia may be associated with modulation of Ca^{2+} release from SR regulated by 1,4,5-trisphosphate cascade.

This study also was shown that the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation in aortic rings precontracted with KCl was significantly attenuated in presence of nifedipine, a specific blocker of VDCCs. These data suggest that the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation is associated with changes in Ca^{2+} influx through VDCCs which is mainly responsible for contraction induced by KCl. This suggestion is consistent with the several studies that have shown that hypoxia-induced vasorelaxation is associated with decreased $[Ca^{2+}]_i$ due to direct inhibition of VDCCs. In addition, hypoxia-induced vasorelaxation may be due to decreased $[Ca^{2+}]_i$ resulting from reduced Ca^{2+} influx via VDCCs mediated by membrane hyperpolarization induced by activation of K_{Ca} and K_{ATP} [22]. In this study, we have found that a specific inhibitor of K_{ATP} channel, glibenclamide, and, a nonspecific inhibitor of BK_{Ca} channel TEA significantly attenuated the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation in aortic rings precontracted with KCl. Conversely, the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation was not significantly reduced by $BaCl_2$, a potent inhibitor of K_{IR} channels. Significant reduction of the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation in the presence of glibenclamide and TEA suggests that K_{ATP} and BK_{Ca} channels participate in the inhibitory action of conjugate DHQ-11.

At the same time, it was shown that an important role in the hypoxia-induced vasorelaxation plays endothelium which produced various vasoactive factors involved in the regulation of vascular tone [23]. Among these factors, a key role in the mechanisms of endothelium-mediated vasorelaxation plays NO that through activation of sGC/cGMP/PKG pathway lead to inhibition of VDCCs and facilitation of Ca^{2+} uptake by Ca^{2+}-ATPase of SR resulted in the decrease of intracellular level of Ca^{2+} ($[Ca^{2+}]_i$). It has been shown that hypoxia decreased the bioavailability of NO through reductions in eNOS activity and thus disrupt of NO/sGC/cGMP/PKG pathway resulting in impaired endothelium-dependent vasorelaxation [24]. In the present study was found that the removal of endothelium significantly reduced the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation in aortic rings precontracted with KCl and PE. Furthermore, in these studies was observed that the removal of the endothelium more markedly reduced the inhibitory effect of the conjugate DHQ-11 on hypoxia-induced vasorelaxation in aortic rings precontracted with PE. This finding suggests that the effect of conjugate DHQ-11 on the hypoxia-induced vasorelaxation is mediated via an endothelium-dependent mechanism possible by involving NO/sGC/cGMP/PKG pathway. To further clarify the significance of the NO/sGC/cGMP/PKG pathway in the inhibitory effect of conjugate DHQ-11 on the hypoxia-induced vasorelaxation its effects on endothelium-intact aortic rings preincubated with L-NAME, methylene blue, and indomethacin, were studied. Because the contractile response to PE is more sensitive to hypoxia than is that to KCl these studies were mainly performed on aortic rings precontracted with PE. In these studies, it was found that preincubation of aortic rings with L-NAME and methylene blue significantly attenuated the inhibitory effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation. In contrast, preincubation of endothelium-intact aortic rings with indomethacin not significantly affected the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation. These results support our suggestion that the effect of conjugate DHQ-11 on hypoxia-induced vasorelaxation is mediated by the endothelium-dependent mechanism involving NOS and NO/sGC/cGMP/PKG pathway.

In conclusion, our study demonstrated that conjugate DHQ-11 exerts a marked protective effect against hypoxic injuries in the vasculature both through endothelium-independent and -dependent mechanisms. The endothelium-independent mechanism is likely involves modulation of VDCCs, K_{ATP}, $BK_{Ca,}$ and IP_3Rs whereas modulation of eNOS and NO/sGC/cGMP/PKG pathway might contribute to the endothelium-dependent mechanism. Further studies of the mechanisms underlying the protective effect of conjugate DHQ-11 may provide new therapeutic strategies for the prevention and treatment of arterial hypertension as well as a wide range of CVDs associated with hypoxia-ischemia.

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