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Mechanism of vasorelaxant effect of the indole alkaloid 12-hydroxynorfluorocurarine hydrochloride on aortic smooth muscle contraction

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ABSTRACT

This article examines the mechanism of action of 12-hydroxynorfluorocurarine hydrochloride on vascular contractile activity. The studies were conducted *in vitro* using rat aortic rings. The isometric force of the contractile force of the rings was measured using an FT-03 force transducer (Grass Instrument, USA). 12-Gydroxynorfluorocurarine hydrochloride produced a dose-dependent vasorelaxant response on phenylephrine-induced aortic ring contractility, with the vasorelaxant response being reduced in the absence of endothelium. The vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride is driven by modulation of the endothelial NO synthase (eNOS), guanylate cyclase (sGC), inward-rectifier K^+ channels (K_{ir}), and Ca^{2+}-activated K^+ channels. Furthermore, when 50 mM KCl is present, this indole alkaloid was observed to diminish the force of aortic contraction induced by elevated Ca^{2+} ion concentration. 12-Hydroxynorfluorocurarine hydrochloride has been shown to exert potent vasorelaxant effects on rat aortic preparations through both endothelium-dependent and endothelium-independent pathways. It has been shown that the vasorelaxant action of 12-hydroxynorfluorocurarine hydrochloride is mediated by blocking L-type Ca^{2+} channels via the eNOS/NO/sGC signaling cascade and by activating K_{ir} channels.

1. INTRODUCTION

Today, worldwide, the incidence and mortality from cardiovascular diseases (CVDs) are rapidly increasing among the population [1–3]. Arterial hypertension has a significant impact on the onset of CVD. One of the primary contributors to the development of arterial hypertension is the disturbance of Ca^{2+} homeostasis in the smooth muscle cells (SMCs) of blood vessels [4,5]. Activation of Ca^{2+} channels in SMCs results in a rise in Ca^{2+} concentration in the cytosol, which causes an increase in vascular tone [6–8]. In this instance, vasorelaxation diminishes because of a reduction in the permeability of K^+ channels. In this state, the cell undergoes depolarization, and the force of contraction of vascular smooth muscles increases as a consequence of the influx of Ca^{2+} ions through L-type Ca^{2+} channels [9,10]. In addition, malfunction of the vascular endothelial layer is a factor in the development of CVDs. In this case, endothelial cells lose the ability to regulate vasorelaxation and vascular tone. Taking this into account, the creation of new drugs that modulate ion channels and restore endothelial

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function is one of the urgent tasks of modern pharmaceuticals [11–14]. Numerous research institutions are employing bioactive compounds extracted from plants to regulate the function of ion channels in vascular SMCs [15,16]. Promising targets include indole, isoquinoline, diterpenoid alkaloids, and flavonoids [17–19]. Consequently, we investigated the mechanisms of action of the indole alkaloid 12-hydroxynorfluorocurarine hydrochloride on the aortic vessel's endothelial layer and several ion transport systems.

2. MATERIALS AND METHODS

2.1. Chemical substances

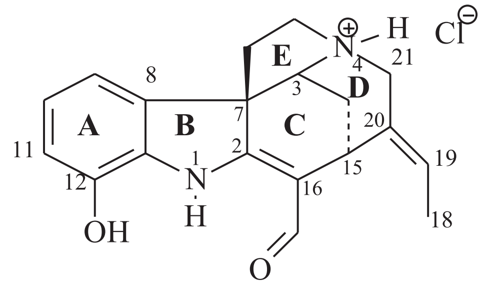
All chemicals were analytical-grade, such as phenylephrine (PE), N^ω-nitro-L-arginine methyl ester (L-NAME), acetylcholine chloride, indomethacin, tetraethylammonium (TEA), 4-aminopyridine (4-AP), glibenclamide, barium chloride ($BaCl_2$), ethylene glycol bis (β-aminoethyl ether) -N,N,N′,N′-tetraacetic acid, nifedipine, and dimethyl sulfoxide (DMSO) (Sigma). Indomethacin, nifedipine, and glibenclamide were dissolved in DMSO. 12-Hydroxynorfluorocurarine hydrochloride and other substances were dissolved in double-distilled water. The highest DMSO concentration in a 5 ml organ bath with rat aortic vascular rings is limited to 0.1% and does not influence smooth-muscle activity.

2.2. Isolation of 12-hydroxynorfluorocurarine hydrochloride

The plant *Vinca erecta*, collected in the mountainous region of the Fergana Valley (Uzbekistan), was used as raw material (5 kg). The plant material was cut into 5–9 mm fragments, moistened with 4% aqueous ammonia, and subjected to percolation in a 10 l percolator with chloroform (10 l) for 12 hours. Following extraction, chloroform was eliminated at reduced pressure with a rotary evaporator. The obtained raw extract was rinsed with distilled water and further processed to yield an alkaloid-rich fraction. This alkaloid mixture was chromatographed on aluminum oxide columns using methylene chloride and acetone as eluents (stepwise elution, 100% CH_2Cl_2 followed by 100% acetone). Under these conditions, 12-hydroxynorfluorocurarine was obtained as an individual alkaloid [20].

Vinca erecta Rgl., native to Central Asia [21], is recognized as an abundant source of indole alkaloids, including norfluorocurarine (vincanine), 12-hydroxynorfluorocurarine (vincanidine), and 12-methoxynorfluorocurarine (vincanicine), which collectively represent about 1% of the plant's dry weight [22]. The crystal structure of water-soluble N^4-methyl derivatives of 12-hydroxynorfluorocurarine was previously reported [23].

12-Hydroxynorfluorocurarine, a derivative of α-methylindole, undergoes acid-catalyzed transformation with HCl to form indolenine (desformylvincanidine), which under specific conditions yields indoline and indole derivatives. Oxidative transformation of the O-methyl ester of desformyl dihydrovincanidine into 4-methoxy-N-oxalyl anthranilic acid validated the location of the phenolic OH group in vincanidine. The structure and absolute configuration of 12-hydroxynorfluorocurarine were determined by the generation of indolenine, indoline, and indole derivatives, in addition to Ultraviolet (UV), Infrared (IR), and mass spectroscopy, and by conversion of tetrahydrovinervine into tetrahydroaquammycin [24]. The hydrochloride salt of 12-hydroxynorfluorocurarine is thus recognized as a vincanidine derivative.



12-Hydroxynorfluorocurarine hydrochloride

2.3. Experimental animals

White male Wistar rats weighing between 200 and 300 g were used in the experiments. The animals were housed in a dedicated room at a temperature of 24°C ± 1°C, with constant access to water and food. They were fed a custom diet formulated to be nutritionally balanced. All procedures for isolating aortic rings were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [25] and the ARRIVE guidelines (https://arriveguidelines.org/). The study protocol was pre-approved by the Ethics Committee of the Institute of Biophysics and Biochemistry, National University of Uzbekistan (protocol no. 7, 7 April 2022).

2.4. Tissue preparation

Wistar rats were anesthetized with sodium pentobarbital (40 mg/kg), and while deeply anesthetized, were ethically euthanized by cervical dislocation followed by decapitation. The thorax was then opened, and the aorta was meticulously isolated. The isolated blood vessel was placed in a special Petri dish, cleared of excess fatty tissue, and aortic preparations measuring 3–4 l~ millimeter were prepared. During the experiments, the endothelial layer of the aortic blood vessel was removed physically using a cotton applicator [26]. The prepared aorta preparation was placed in a cell containing Krebs solution: (mM) NaCl, 118; KCl, 5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2; $NaHCO_3$, 25; glucose ,11 (pH = 7.4).

2.5. Aortic-ring contraction studies

The aortic rings were attached to two stainless-steel hooks, one of which was secured to the base of the organ bath while the other was linked to the force transducer hook. The aortic annulus was infused with a continuous Krebs solution and oxygenated with 95% O_2 and 5% CO_2. The aortic ring was kept in Krebs solution under a tension of 1 g (~10 mN) for 60 minutes. The force of contractile of the aortic ring in isometric conditions was measured using a specialized

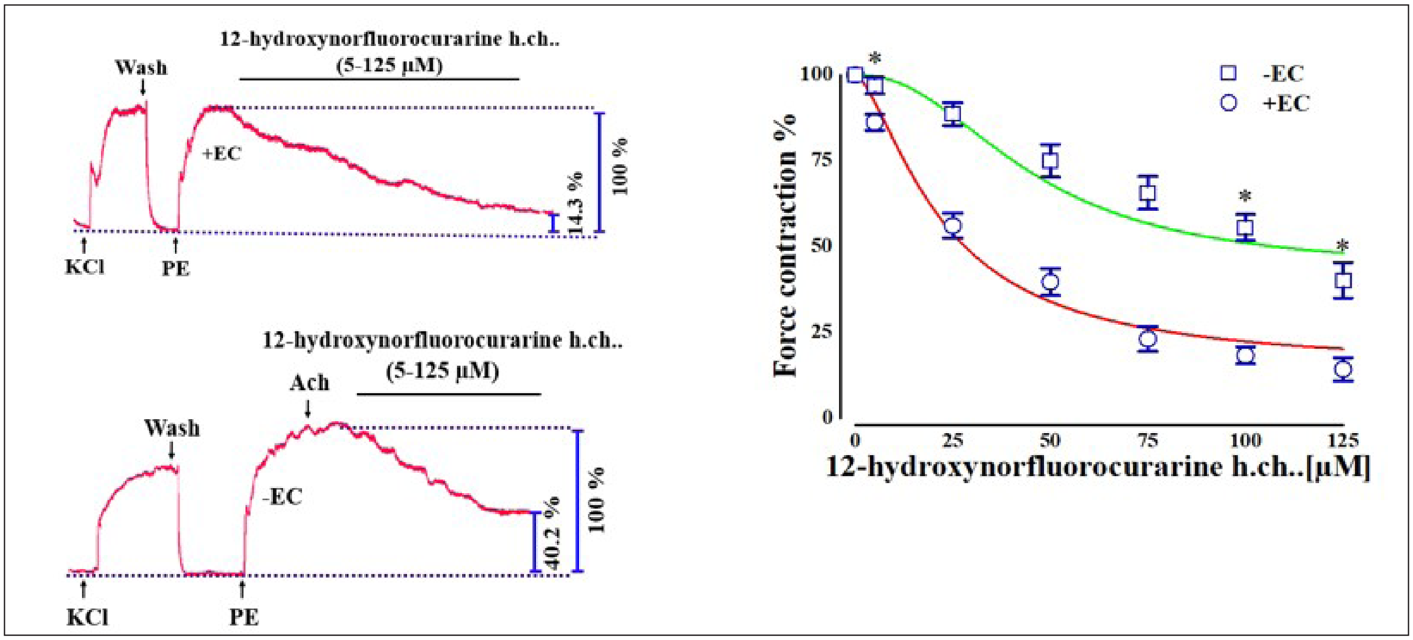


Figure 1. (A) Vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on aortic rings. (B) Dose-dependent vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on the contractile force of rat aortic rings induced by PE (1 μM) under endothelium-intact (+EC) and endothelium-denuded (−EC) conditions. The data are expressed as the means ± SEM of five rats (n = 5) (one-way ANOVA followed by Dunnett's post hoc test, $*p < 0.05$ vs. control group; $^{\#}p < 0.05$ compared between the treated groups).

device (FT-03; Grass Instrument). Temperature constancy (t = 37°C ± 0.5°C) was ensured by the U–8 ultra-thermostat. The mechanisms underlying the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on aortic ring contractions were investigated.

2.6. Experimental protocols

Vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on the aortic ring. In preliminary experiments, aortic rings were induced to contract with KCl (50 mM) and PE (1 μM), and the dose-dependent (5–125 μM) vasorelaxant effects of 12-hydroxynorfluorocurarine hydrochloride were investigated. In certain experiments, the endothelial layer of aortic rings was excised, and the effect of 12-hydroxynorfluorocurarine hydrochloride was studied.

The effect of 12-hydroxynorfluorocurarine hydrochloride on vascular endothelial function to assess the role of the endothelium in the vasorelaxant action of 12-hydroxynorfluorocurarine hydrochloride on aortic rings, the dose-dependent relaxation was examined in rings whose endothelial layer had been mechanically stripped. In addition, the inhibitor L-NAME (100 μM) was employed to evaluate the effect of 12-hydroxynorfluorocurarine hydrochloride on endothelial NO synthase (eNOS), indomethacin (10 μM) was used to assess the effect on cyclooxygenase (COX), and methylene blue (10 μM) was applied to test the effect on guanylate cyclase (sGC). In this case, the contraction was induced by PE, and the dose-dependent vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride was studied under incubation conditions of these blockers.

Table 1. Vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on endothelium-containing and endothelium-free aortic preparations. IC_{50} and Hill coefficient.

12-hydroxynorfluorocurarine hch..	$\%E_{min}$	$\%E_{max}$	IC_{50}	h
Endothelium-intact (+EC)	13.8% ± 2.3%	85.7% ± 3.4%	22.5 ± 3.0	1.5 ± 0.2
Endothelium-denuded (−EC)	2.4% ± 1.6%	59.8% ± 5.1%	57.2 ± 5.3	2.2 ± 0.4

The values are expressed as means ± SEM, n = 5.

Effect of 12-hydroxynorfluorocurarine hydrochloride on K^+ channels. In these experiments, the role of K^+ channels in the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride was investigated. In this study, aortic rings from which the endothelial lining was stripped were incubated with various K^+ channel blockers and then contracted with PE (1 μM). Including: under incubation conditions with the Ca^{2+}-activated K^+ channels (K_{Ca})-TEA blocker (5 mM), voltage-dependent K^+ channels (K_V)-4-AP blocker (1 mM), ATP-responsive K^+ channels (K_{ATP})-glibenclamide blocker (10 μM), inward-rectifier K^+ channels (K_{ir})-$BaCl_2$ blocker (1 mM), 12-hydroxynorfluorocurarine hydrochloride was cumulatively added from 5–125 μM. The results gathered were compared with the control group.

Effect of 12-hydroxynorfluorocurarine hydrochloride on Ca^{2+} concentration. To study the effect of 12-hydroxynorfluorocurarine hydrochloride on L-type Ca^{2+} channels, physiological Krebs solution with a range of $[Ca^{2+}]_{out}$ = 0.5–2.5 mM was used. In this case, under

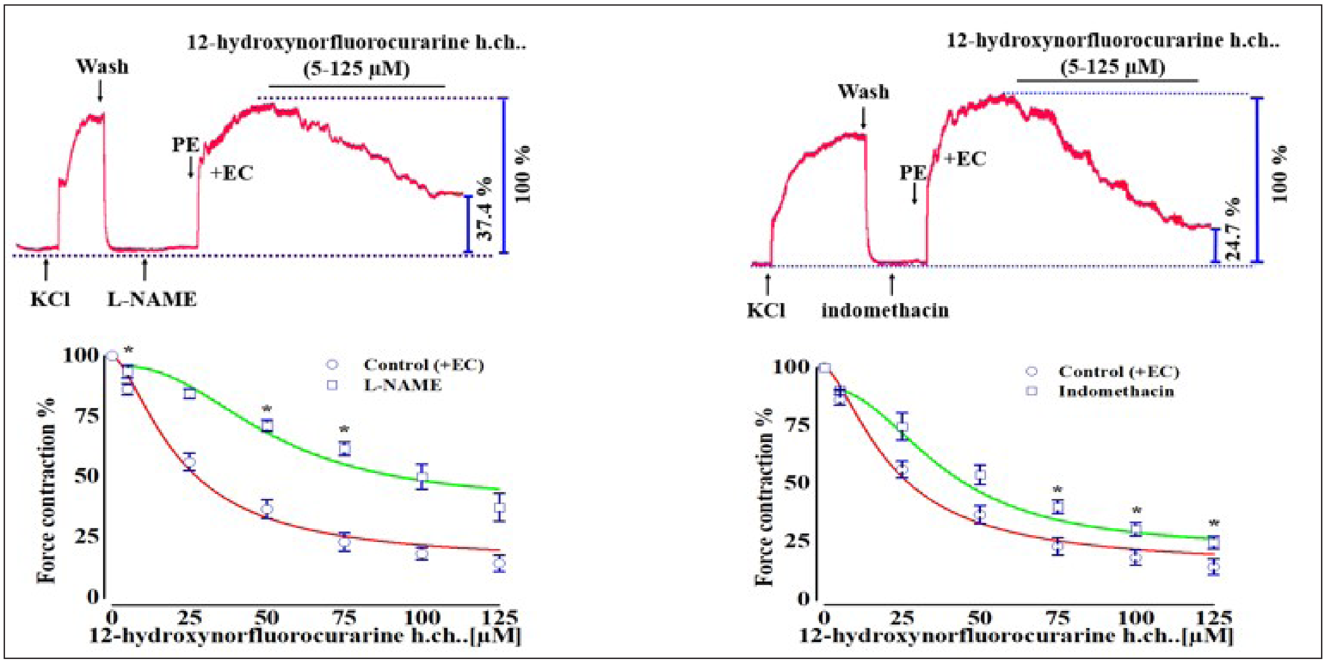


Figure 2. (A) Effect of 12-hydroxynorfluorocurarine hydrochloride on NO and COX. (B) Graphic representation of the effect of 12-hydroxynorfluorocurarine hydrochloride on the force of aortic contraction induced by PE in the presence of L-NAME. Graphic representation of the effect of 12-hydroxynorfluorocurarine hydrochloride on the force of aortic contraction induced by PE in the presence of indomethacin. The data are expressed as the means ± S.E.M. of five rats (n=5). (Unpaired t-test, *p < 0.05 vs. control group).

Table 2. Vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride. Effect of eNOS and COX inhibitor (%E_{min} and E_{max}) on the magnitude of contraction induced by PE 1 µM. IC_{50} and Hill coefficient.

Groups	%E_{min}	%E_{max}	IC_{50}	h
Control	13.8% ± 2.3%	85.7% ± 3.4%	22.5 ± 3.0	1.5 ± 0.2
L-NAME	6.5% ± 2.7%	62.6% ± 5.6%	59.0 ± 3.8	2.8 ± 0.5
Indomethacin	9.6% ± 0.4%	75.3% ± 2.6%	35.9 ± 3.5	2.0 ± 0.3

The values are expressed as means ± S.E.M., n=5.

incubation conditions with KCl, an increase in the force of muscle contraction was noted due to a rise in the concentration of Ca^{2+} ions in the medium [27]. Pre-addition of 12-hydroxynorfluorocurarine hydrochloride reduced this contractile strength compared to control. Experiments were additionally performed with the L-type calcium channel antagonist nifedipine. The results obtained were compared, and the differences in the strength of influence were also compared.

2.7. Statistics

During the *in vitro* experiments, the contractile force of the SMCs (measured in mN) in the aortic preparations was converted into percentage values for statistical analysis. Data processing was carried out using Origin 8 (OriginLab Corporation, Northampton, MA). The normality of the data distribution was assessed using the Shapiro–Wilk test. Outliers were identified based on Chauvenet's criterion and excluded where appropriate. The results are presented as the mean ± SEM across n observations. For multiple group comparisons, ANOVA was employed, followed by a Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$. The goodness of fit for curve fitting was evaluated using the adjusted R^2 values generated by the Origin 8 fitting algorithm.

In addition, the Hill–Langmuir equation was used to model the vasorelaxant responses of the test compounds, expressed as follows:

$$E = \frac{E_{\max}\left(\frac{C}{EC_{50}}\right)^n}{1+\left(\frac{C}{EC_{50}}\right)^n}$$

Here, E is the magnitude of the vasorelaxant effect relative to contractile force, E_(max) is the maximal vasorelaxation relative to contraction, and EC_{50} (or IC_{50}) is the concentration that elicits half of the maximal effect (i.e., reduces contractile force by ~50%). n is the Hill coefficient [28].

3. RESULTS

3.1. Vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on rat aortic ring

In the initial investigations, 12-hydroxynorfluorocurarine hydrochloride was observed to lower the PE-induced contractile force (1 µM) in rat

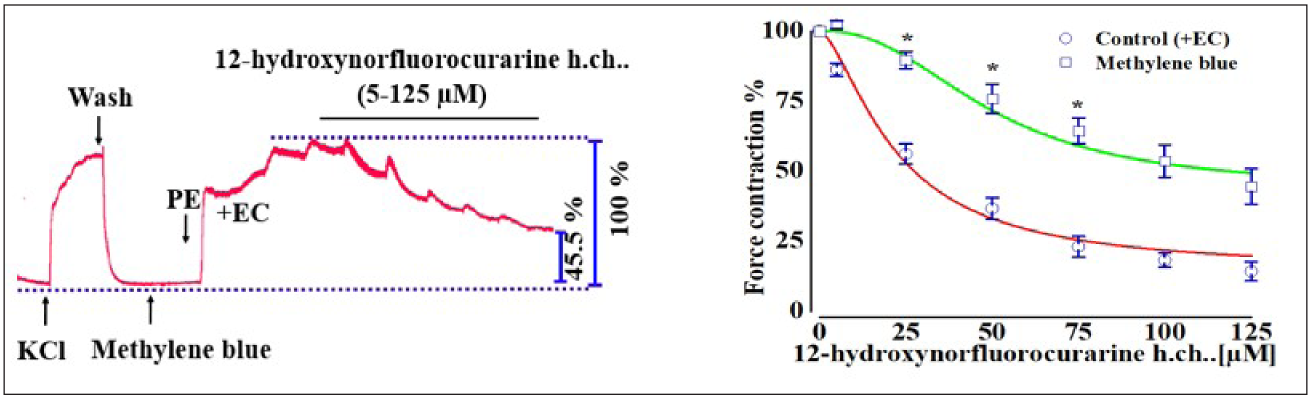


Figure 3. (A) Involvement of sGC in the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride. (B) Effect of 12-hydroxynorfluorocurarine hydrochloride on PE-induced aortic annular contractility in the presence of methylene blue. The data are expressed as the means ± S.E.M. of five rats ($n = 5$). (Unpaired *t*-test,$^*p < 0.05$ vs. control group).

Table 3. Vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride and the effect of an sGC inhibitor (%E_{min} and E_{max}) on contraction induced by 1 μM PE. IC_{50} and Hill coefficient.

Groups	%E_{min}	%E_{max}	IC_{50}	*h*
Control	13.8% ± 2.3%	85.7% ± 3.4%	22.5 ± 3.0	1.5 ± 0.2
Methylene blue	2.1% ± 1.5%	55.5% ± 3.3%	51.0 ± 3.8	2.3 ± 0.4

The values are expressed as means ± S.E.M., $n = 5$.

aortic rings by 13.8% ± 2.3 % at 5 μM and by 85.7% ± 3.4 % at 125 μM (Fig. 1A and B). In subsequent studies, experiments were performed on endothelium-free aortic rings, in which a 59.8% ± 5.1% reduction in the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride (125 μM) on aortic contractile force was observed (Fig. 1A and B). The resulting concentration of DMSO was below 0.1% and did not influence vascular smooth muscle contraction and relaxation. These results indicate that the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride is inextricably linked to the endothelium. Also, the IC_{50} value of the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride was IC_{50} = 22.05% ± 1.54% μM in the preparation with preserved endothelium and 45.2% ± 2.25% μM in the absence of endothelium. The Hill coefficient (*h*) was 1.51 ± 0.2 and 2.2 ± 0.3, respectively (Fig. 1B, Table 1).

3.2. The role of the endothelium in the vasorelaxant action of 12-hydroxynorfluorocurarine hydrochloride

Given that the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride in previous experiments was endothelium-dependent, further experiments were carried out in the presence of the eNOS inhibitor L-NAME. It was observed that the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride (125 μM) on the contractile force induced by PE (1 μM) in the presence of L-NAME (100 μM) in the medium decreased from 85.7% ± 2.8 % to 62.6% ± 5.6 %. The following experiments were performed out in the presence of the COX inhibitor indomethacin. Furthermore, the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on the force of contraction of the aortic ring induced by PE (1 μM) in the presence of indomethacin (10 μM) in the bath was reduced by 10.3% ± 2.7%. The findings suggest that the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride stems from a modest involvement of COX, while the principal contribution comes from the eNOS signaling system (Fig. 2A and B, Table 2).

3.3. Involvement of sGC in the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride

The vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride may also involve sGC via the eNOS signaling pathway. To evaluate the participation of sGC, the force of aortic annular contraction triggered by PE (1 μM) in the presence of endothelium was decreased by 12-hydroxynorfluorocurarine hydrochloride from 85.7% ± 3.4 % to 55.5% ± 6.3 % when the sGC inhibitor methylene blue (10 μM) was applied. These results suggest that the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride could be mediated through the stimulating of sGC, thereby activating the synthesis of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) (Fig. 3A and B, Table 3).

3.4. Effect of 12-hydroxynorfluorocurarine hydrochloride on K^+ channels

To assess the role of K^+ channels in the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride, experiments were conducted on endothelium-denuded aortic rings, in the presence of the K_{ATP} channel blocker glibenclamide (10 μM), the K_V channel blocker 4-AP (1 mM), the K_{Ca} channel blocker TEA (5 mM), and the K_{ir} channel blocker $BaCl_2$ (1 mM). In experiments, the aortic ring, where the endothelium had been stripped, when exposed to $BaCl_2$ (1 mM), diminished the contractile force triggered by PE (1 μM) from 57.1% ± 2.5 % to 15.5% ± 2.3 %. In the next experiment, it was observed that the studied alkaloid decreased the contractile force elicited by PE (1 μM) from 57.1% ± 2.5% to 42.6% ± 3.5% when TEA (5 mM) was present in the medium. Moreover, the experiments demonstrated that the vasodilatory effect of 12-hydroxynorfluorocurarine

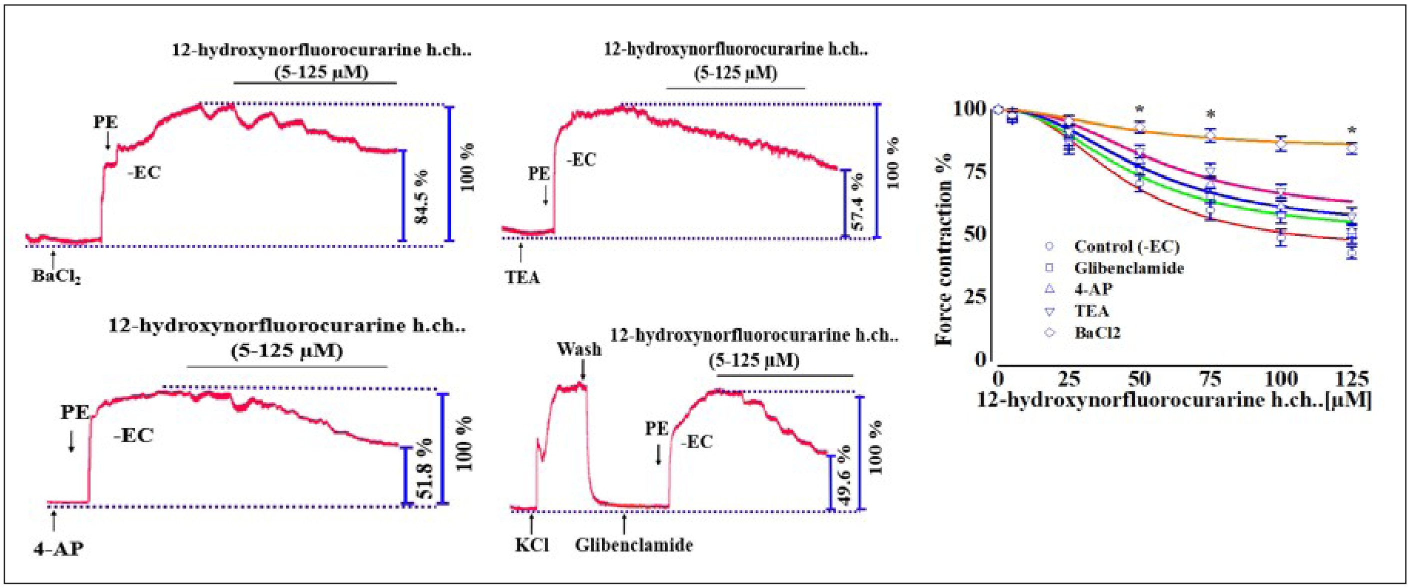


Figure 4. (A) Effect of 12-hydroxynorfluorocurarine hydrochloride on the contractile force of rat aortic rings induced by PE in the presence of K^+ channel blockers. (**B**) The ordinate axis represents the contractile force of the aortic preparation as a percentage, with the contractile force induced by 1 μM PE taken as 100%. The data are presented as the means ± S.E.M. of five rats ($n = 5$). (One-way ANOVA followed by Dunnett's post hoc test, $*p < 0.05$ vs. control group).

Table 4. Effect of 12-hydroxynorfluorocurarine hydrochloride on vasorelaxation indices (%E_{min} and E_{max}) in aortic endothelial rings induced by PE (1 μM) in the presence of K^+ channel blockers. IC_{50} and Hill coefficient.

Groups	%E_{min}	%E_{max}	IC_{50}	h
Control (EC-)	2.4% ± 1.9%	57.1% ± 2.5%	57.2 ± 5.3	2.2 ± 0.4
Glibenclamide	2.05% ± 1.7%	50.4% ± 2.9%	47.6 ± 4	2.1 ± 0.3
4-AP	3.7% ± 1.4%	48.2% ± 2.8%	52.7 ± 5.2	2.2 ± 0.4
TEA	1.3% ± 1.6%	42.6% ± 3.5%	57.85 ± 5.2	2.3 ± 0.5
$BaCl_2$	2.4% ± 1.5%	15.5% ± 2.3%	45.9 ± 6.3	1.9 ± 0.5

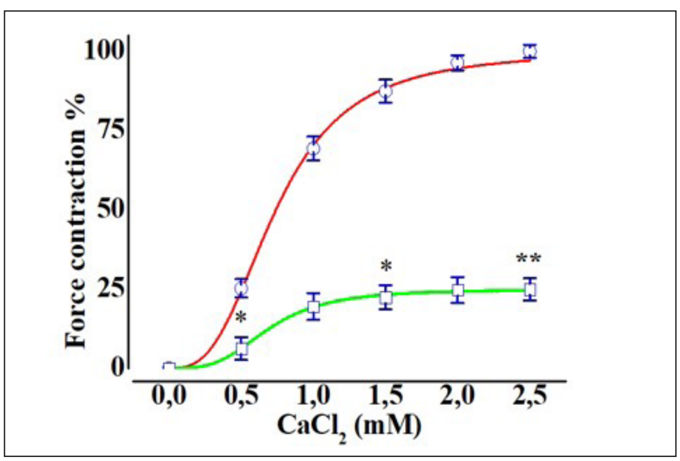


Figure 5. Vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on KCl (50 mM)-induced contractile activity in rat aortic ring, dependent on Ca^{2+} concentration. The contraction force induced by 50 mM KCl was taken as the control (100%). The data are presented as the means±S.E.M.of five rats ($n=5$). (One-way ANOVA followed by Dunnett's post hoc test, $*p<0.05$ vs. control group).

hydrochloride on PE-induced contractile force was not markedly diminished by incubation with 4-AP (1 mM) and glibenclamide (10 mM) (Fig. 4A and B, Table 4).

The experimental results indicate that the vasorelaxing effect of 12-hydroxynorfluorocurarine hydrochloride is due to modulation of the function of K_{Ca} and K_{ir} channels.

3.5. Effect of 12-hydroxynorfluorocurarine hydrochloride on L-type Ca^{2+} channels

The decrease in the force of aortic annular contraction caused by KCl 12-hydroxynorfluorocurarine hydrochloride may be due to a decrease in $[Ca^{2+}]_i$ due to inhibition of L-type Ca^{2+}–channel activation. To elucidate this problem, experiments were performed with changes in the composition of the Krebs–Henseleit physiological solution in the range $[Ca^{2+}]_{out}$ = 0.5–2.5 mM. Increasing the concentration of Ca^{2+} ions in the Krebs solution increased the force of aortic contraction depending on the dose of KCl (50 mM). Under these conditions, 12-hydroxynorfluorocurarine hydrochloride was found to reduce Ca^{2+} concentration-dependent aortic contraction. 12-Hydroxynorfluorocurarine hydrochloride diminished the strength of aortic contraction by 79.9% ± 2.0% under conditions of $[Ca^{2+}]_{out}$ = 2.5 mM. This has been shown to occur through by blocking voltage-gated L-type Ca^{2+} channels. To further elucidate this issue, studies were conducted in the presence of the L-type Ca^{2+}-channel antogonist nifedipine (Fig. 5).

In these experiments, the half-maximal effective concentration of nifedipine, IC_{50} = 0.1 μM, was used. The effect of 12-hydroxynorfluorocurarine hydrochloride (IC_{50} = 56.4 μM) on the contraction of the aortic annulus induced by KCl (50 mM) was studied in the presence of 0.1 μM nifedipine in the incubation medium. It was observed that 12-hydroxynorfluorocurarine hydrochloride decreased the force of contraction of the aortic ring by an additional 18.8% ± 3.7%. These results suggest

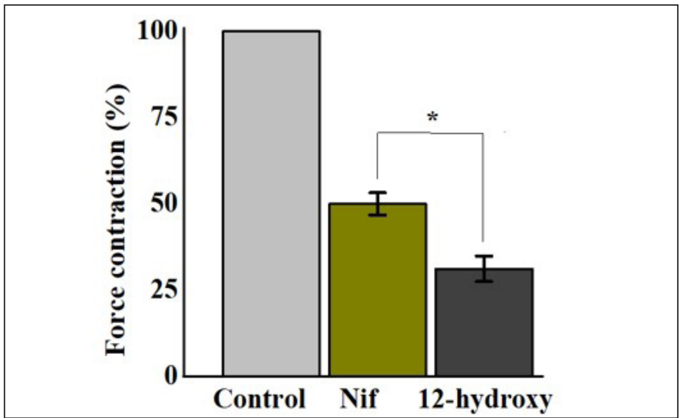


Figure 6. Effect of 12-hydroxynorfluorocurarine hydrochloride on contractile activity induced by KCl (50 mM) in rat aortic rings under incubation with the L-type Ca^{2+}-channel blocker nifedipine (IC_{50}=0.1 μM). The contraction force induced by KCl (50 mM) was taken as control (100%). The data are expressed as the means±S.E.M. of five rats (n=5). (One-way ANOVA followed by Dunnett's post hoc test, *$p < 0.05$ vs. control group).

that 12-hydroxynorfluorocurarine hydrochloride produces its vasorelaxant effect by blocking L-type Ca^{2+} channels, but additional experiments involving. Nifedipine also indicates that it will also affect other ion channels (Fig. 6).

4. DISCUSSION

In this study, the underlying mechanisms of the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on rat aortic rings were examined. In the course of experiments, it was found that 12-hydroxynorfluorocurarine hydrochloride significantly reduces the force of contractile of the aortic preparation evoked by PE. However, eliminating the vascular endothelial layer led to a reduction in the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride. It is recognized that the primary endothelium-dependent relaxation factor is NO synthase [29–31]. NO, synthesized from L-arginine with the participation of eNOS, diffuses into SMCs and activates sGC. As a result, GTP is converted to cGMP, which activates protein kinase G, stimulating smooth muscle relaxation [32–34]. In studies, inhibition of NO synthesis with L-NAME markedly reduced the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride, indicating the importance of the eNOS/NO pathway. In addition, another important endothelium-dependent relaxing factor is the Prostacyclin (PGI_2) pathway. PGI_2 is an endothelium-dependent relaxing factor produced from arachidonic acid by the COX enzyme, which attaches to PGI_2 receptors found on SMCs. Activation of receptors results in a rise in the intracellular concentration of cyclic adenosine monophosphate (cAMP). Increased levels of cAMP and cGMP promote vasorelaxation of SMCs and reduce vascular tone [35–37]. In experiments, the vasorelaxant activity of the indole alkaloid 12-hydroxynorfluorocurarine hydrochloride on aortic contraction induced by PE was not reduced compared to the control under conditions of incubation with the COX enzyme inhibitor indomethacin. Thus, the relaxant effect of the studied indole alkaloid depends on the endothelium and is mediated by the NO/sGC/GTP/cGMP/PCG cascade reaction. To further elucidate this issue, experiments were conducted using the sGC enzyme inhibitor methylene blue. sGC is known to act as a catalyst in the formation of cGMP from GTP and induce vasorelaxation [38–40]. Studies have shown that the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on PE-induced aortic contraction was decreased in the presence of methylene blue. This result confirms that the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride is facilitated by the eNOS/NO/sGC signaling pathway.

K^+ channels have a pivotal role in modulating the functional activity of vascular SMCs. Activation of K^+ channels result in hyperpolarization, decreased $[Ca^{2+}]_{in}$ and vasodilation, whereas their inhibition results in depolarization, increased $[Ca^{2+}]$ in and vasoconstriction. Four categories of K^+ channels exist in vascular smooth muscle: K_{ir}, K_{ATP}, K_V, and K_{Ca} [41–44]. All of these channels have been shown to alter the physiological state of vascular smooth muscles under the influence of biologically active compounds [45,46]. Therefore, to study the role of K^+ channels in the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on contractions of the aortic vessels, the specific K_{ATP} channel inhibitor glibenclamide, the K_V channel antagonist 4-AP, the K_{Ca} channel blocker TEA, and the K_{ir} channel blocker $BaCl_2$ were used. In experiments, preincubation with $BaCl_2$ and TEA markedly reduced the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride on PE-induced contractions of the aortic preparation. In this case, $BaCl_2$ reduced the relaxant effect of the indole alkaloid more strongly than in the presence of TEA. Also, incubation with 4-AP blockers and glibenclamide did not produce a notable effect on the vasorelaxant action of 12-hydroxynorfluorocurarine hydrochloride. These results suggest that K_{ir} channels play a more important role than other K^+ channels in the relaxant effect of 12-hydroxynorfluorocurarine hydrochloride.

The indole alkaloid 12-hydroxynorfluorocurarine hydrochloride was observed to produce a strong vasorelaxant effect on the magnitude of aortic contraction induced by KCl (50 mM). It is known that extracellular Ca^{2+} ions are transported into the cytosol via voltage-gated L-type Ca^{2+} channels situated in the plasma membrane of SMCs [47,48]. Thus, the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride may be due to blockade of L-type Ca^{2+} channels. In experiments, when the concentration of Ca^{2+} ions in a 50 mM Krebs KCl solution changed to 0.5–2.5 mM, the contraction force increased. 12-Hydroxynorfluorocurarine hydrochloride was shown to significantly reduce the force of aortic contraction induced by an increase in Ca^{2+} ion concentration. This indicates that the vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride is mediated through inhibition of L-type Ca^{2+} channels. However, the additional relaxation of 12-hydroxynorfluorocurarine hydrochloride during KCl-induced aortic contraction when the L-type calcium channel blocker nifedipine suggests that other calcium channels are involved in this process.

5. CONCLUSIONS

These studies demonstrate that 12-hydroxynorfluorocurarine hydrochloride exerts potent vasorelaxant effects on rat aortic preparations via endothelial-dependent and other mechanisms. The vasorelaxant effect of 12-hydroxynorfluorocurarine hydrochloride is facilitated by activation of the eNOS/NO/sGC cascade pathway, and effects are consistent with involvement of K_{ir} channels, likely blockade of Ltype Ca^{2+} channel activity. These results may explain the underlying mechanism of vasorelaxation associated with 12-hydroxynorfluorocurarine hydrochloride observed in *in vitro* studies. Nevertheless, *in vivo* studies ought to be conducted in subsequent investigations.

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7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

All procedures for isolating aortic rings were conducted in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the ARRIVE guidelines (https://arriveguidelines.org/). The procedures were also pre-approved by the Ethics Committee of the Institute of Biophysics and Biochemistry at the National University of Uzbekistan (protocol no. 7, dated 7 April 2022).

11. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

12. PUBLISHER'S NOTE

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13. USE OF ARTIFICIAL INTELLIGENCE-ASSISTED TECHNOLOGY

All authors confirm that no artificial intelligence (AI) tools were used to draft or revise this manuscript, and that no images were modified using AI.

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