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The Roles of Potassium Channels and Nitric oxide in the Modulation of Apelin induced-relaxation in Isolated Diabetic Rat Aorta

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Abstract

Diabetes is associated with endothelial dysfunction, which impairs blood vesselscapacity to maintain vascular tone. Apelin is an adipocyte-produced relaxing factor that has endothelium-dependent and nitric oxide (NO)-mediated vasorelaxant effects. The current study investigated how streptozotocin (STZ)-induced type one diabetes modulates the mechanisms involved in aortic vascular response to apelin, focusing on the role of potassium channels and endothelial derived relaxing factors (EDRF). In this study, precontracted rat thoracic aortic segments were pre-incubated with the NO inhibitor, cyclooxygenase (COX) inhibitor and potassium channels blockers including: non-selective calcium-activated potassium channel, big conductance calcium-activated potassium channels (BKca), intermediate conductance calcium activated potassium channels (IKca), delayed inward rectifier potassium channels (Kir), adenosine triphosphates-sensitive potassium channels (KATP) and voltage sensitive potassium channels (Kv) blockers, then cumulative concentrations of apelin were applied to each group in both non-diabetic and diabetic conditions. The statistical analysis between diabetic and non-diabetic groups revealed that endothelial impairment induced by diabetes in rat thoracic aorta remarkably attenuated the vascular responses to apelin. An important new finding in this study was that almost all potassium channels blockers noticeably $P<0.001$ increased apelin efficacy with relatively no changes in the peptide potency. However, in diabetic aortic segments, the non-selective K_{ca}, BKca blockers, NO inhibitor and COX inhibitor reversed vascular responses to apelin, but Kir, K_{ATP} and Kv blockers significantly reduced vascular responses to apelin in comparison to control rats. It is worth noting that diabetes did not only alter the peptide potency in these experimental groups but also significantly increased the maximum responses when K_{ca} and BKca blockers preincubated. In conclusion, our findings shed light on the mechanisms behind diabetes-induced aortic artery hypo-reactivity to apelin which involve the inhibition of endothelial NO synthase activities and decreased contribution of K_{ca}, BK_{ca}, IK_{ca}, K_v, Kir and K_{ATP} channels.

Keywords: Apelin, Diabetes, Endothelial dysfunction, Potassium channels

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دور قنوات البوتاسيوم و اوكسيد النايتريك في تعديل استرخاء شرايين الابهر المحفز بالـ Apelin والمعزولة من الجرذان المصابة بداء السكري المستحث

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الخلاصة

هناك علاقة بين الداء السكري والخلل الوظيفي في البطانة الاوعية الدموية حيث يضعف قدرة الاوعية على شدتها العضلية. ابيلين (Apelin) هو هورمون وعامل مساعد في استرخاء العضلات الملساء ويعتمد على ميكانيكية اوكسيد النتريك (NO) . تستهدف الدراسة الحالية كشف تاثير ابيلين Apelin على استرخاء عضلات الملساء في الجرذان المصابة بداء السكري المستحث مع التركيز على دور قنوات البوتاسيوم وعوامل الاسترخاء الاخرى من ضمنها NO. تضمنت الدراسة الحالية شرائح الابهر الصدري للجرذان وتم استخدام مثبطات قنوات البوتاسيوم المختلفة كما يلي: التوصيل الكبير (BK_{ca+2})، ذات التوصيل المتوسط (IK_{ca+2})، قناة البوتاسيوم من نوع (K_{ir})، قناة البوتاسيوم ذات حساسية لل ATP (K_{ATP}) وذات حساسية للجهد الكهربائي (K_v). بعدذلك، تم استخدام التراكيز التراكمية لهرمون ابيلين Apelin على كل من مجموعة المسيطرة (Control) والمجموعة المستحثة بداء السكرى. اظهرت النتائج بأن استجابة الاوعية الدموية لهورمون ابيلين Apelin انخفضت بشكل معنوي في الجرذان المستحثة بداء السكري مقارنة بمجموعة السيطرة، هذا الاكتشاف الجديد قد يتعلق بقنوات البوتاسيوم وخاصة Bk_{ca+},K_{ca+} و مثبط NO (L−NAME) ومثبط انزيمات الاكسدة الحلقية, COX (Indomethacin), وبعكس هذا فقد قللت مثبطات K_{ir}, K_{ATP}, K_v و بشكل ملحوظ من استجابة الاوعية الدموية لهرمون ابيلين Apelin مقارنة بجرذان مجموعة السيطرة. وتجدر الاشارة الى انه في الجرذان المستحثة بداء السكري لم يغير المرض من فاعلية الهرمون ابيلين Apelin وحسب ، بل زاد و بشكل ملحوظ من الاستجابة القصوى عند الحضن المسبق مع كل من مثبطات K_{ca+}و Bk_{ca+} . توصلت النتائج الى كشف الية عمل هرمون ابيلين Apelin خلال استرخاء العضلات الملساء في الشريان الابهري حيث يتواسط هذه الالية كل من NO، K_{ca+}، BK_{ca+}، IK_{ca+}، K_v، K_{ir} و K_{ATP}.

Introduction

Diabetes mellitus is a prolonged metabolic disorder characterized by elevated blood glucose levels [1]. Studies have indicated that shortly after the onset of diabetes development, endothelial dysfunction with disruption of endothelium-dependent relaxation and hyperpolarization of vascular smooth muscle cells (VSMC) appear in both small and large blood vessels [2, 3] [4]. Vascular homeostasis depends on the capacity of endothelium to release EDRFs, like NO and COX-metabolites, as well as vasoconstrictors such as ET-1 and thromboxane A2 [5]. Hyperpolarization of VSMCs by the outward flow of potassium ions via the opening of K_{Ca}, K_V, K_{ATP} and K_{ir} channel isoforms is a further way for the endothelium to modify their tones [6, 7]. Reduced NO bioavailability due to insulin production impairment and hyperglycemia is identified as a typical putative mechanism for diabetes-induced endothelial dysfunction. Indeed, considerable evidence supports the notion that hyperglycemia limits NO bioavailability through the formation of advanced glycation end products [8]. High endothelin-1 (ET-1) expression, on the other hand, has been identified as an additional prominent feature of diabetes-induced endothelial dysfunction [9].

Apelin is a vasoactive adipokine that has been identified as an endogenous ligand for the apelin–angiotensin receptor-like 1 (APJ) orphan G-protein-coupled receptor [10]. It is generally held that apelin exhibits a direct vasodilator in a variety of tissues with intact arteries [11].

Studies by Jia *et al*. [12] and Yu *et al*.[13] clarified that relaxing effect of apelin in mesenteric artery was attenuated by Nitro-arginine methyl ester (L-NAME), thus suggesting that the vasodilation effect of apelin is mediated at least partly by NO. In VSMC, the relationship between apelin and potassium channels is still unclear. Recently, the inhibition of K_{ca} channels with iberiotoxin has been reported to abolish the relaxation effect of apelin demonstrated by researchers working on rat coronary artery. Mughal *et al.* [14] suggested that involvement of these channels might be the mechanism apelin-associated relaxing effects. However, research in the VSMC of cerebral arteries found that apelin has ability to block BK_{Ca} channel via a PI3-kinase-dependent signaling pathway, possibly contributing to its regulatory role in vascular tone regulation [15].

Although apelin has the potential to cause relaxation in different vascular beds under physiological conditions, studies on apelin effect in diabetic vasculature, as well as how potassium channels and EDRF affect the vascular response to this peptide during the diabetic state, is extremely limited. Accordingly, this approach was designed for the first time to study the direct links between the role of potassium channels and NO in impaired endothelial function induced by diabetes and changes in myogenic tone as well as vascular reactivity of apelin.

Materials and Methods

Animals

This study was carried out on aortic tissues isolated from 10-weeks-old male albino rats weighing between 140-160 gm. Animals were kept under about 12:12 hours light: dark cycle at a temperature of 23 ± 2°C and had access to food and water ad libitum. The experimental protocol was approved by the ethics committee and animal committee in the College of Science, Salahaddin University, Erbil. The research was conducted in the Depatment of Biology, College of Science from March/ 2019 to August / 2019.

Induction of Experimental Diabetes

To induce type one diabetes model in male albino rats, they were given a single intraperitoneal injection of 40 mg/kg streptozotocin dissolved in 0.1 M citrate buffer, pH 4.5 (0.1 mol/l trisodium citrate, 0.1 mol/l citric acids). Three days later, the experimental rats were tested for their fasting blood glucose concentration, with a range of more than 300 mg/dL considered diabetic. The diabetic animals were experimented upon after four weeks of diabetes mellitus induction.

Preparation of Aortic Rings and Tension Measurement.

The thoracic aorta was isolated from rats anesthetized with a ketamine/xylazine mixture (90 mg/kg i.p. and 10 mg/kg i.p., respectively) and placed in an icy-oxygenated Krebs bicarbonate solution comprising the following ingredients in Mm/L: 119 NaCl, 4.7 KCl, 1.2 KH_2 PO_4; 1.2 $MgSO_4$, 1.5 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose, pH = 7.4. Each aorta was cleansed carefully of adhering connective tissues and fat while maintaining endothelial integrity before being cut into four rings (3mm in length). Each segment was then attached between two stainless hooks in an organ bath (Automatic organ bath-Panlab Harvard apparatus-USA, AD Instrument Power Lab 8/35 Australia) with Krebs bicarbonate solution bubbled with a mixture of around 95% O_2 and 5% CO_2. The ring tension was manually adjusted to 2 g and equilibrated for roughly 60 minutes, with the containers being cleaned every 15 minutes with fresh Krebs solution. Subsequently, the vessel viability was checked by using (60 Mm) and maximum contraction developed was considered as standard percentage contractile response. After maximum contraction reached to plateau, the aortic segments were washed several times and were later allowed to equilibrate for 30 minutes before the experimental protocol began.

Experimental Design

Cumulative concentration-response relationships for the relaxant effects of apelin-13 were determined in control and diabetic aortic segments following steady contraction with ET-1 (10^{-7} M).

To investigate the contribution of potassium channels in apelin induced relaxation, the non-diabetic and diabetic aortic segments were preincubated with potassium channel blockers, including the K_v blocker 4-aminopyridine (4-AP) 0.5 mM, the K_{ATP} blocker glibenclamide 10 μM, the K_{ir} blocker barium chloride $BaCl_2$ 0.2 mM, BKca blocker charybdotoxin (chtx) 1 μM, the K_{Ca} blocker clotrimazole (30 μM), and non-selective Kca blocker ; tetraethyl ammonium (TEA) 1mM for 20 min before contraction was induced by ET-1. Cumulative apelin doses were later on added to the organ bath.

To investigate whether endothelial NO was involved in the relaxant effects of apelin, aortic rings were incubated with L-NAME (an irreversible endothelial nitric oxide synthase inhibitor, 200 μM in both non-diabetic and diabetic groups. And to verify the possible role of prostanoids in the apelin relaxant effect, rings were incubated for 20 min before precontraction with ET-1 10^{-7} Mm with a non-selective COX inhibitor indomethacin μM.

Statistical Analysis

The vascular relaxation induced by apelin was expressed as a percentage of tension generated by ET-1. To differentiate the effects of blockers in both non-diabetic and STZ-induced diabetic groups of aortic segments. all results were expressed as the differences area under curves (dAUC). Means ± standard error of the mean (SEM) was used for the expression of all data. Independent students t-test was applied for comparing dAUC and potency difference (pD2) between groups. Two-way analysis of variance (ANOVA) was applied to know the difference, followed by Sidak multiple comparison tests as an individual mean comparison. Dunnett-test was also applied to compare the studied groups with the control and Tukey-test for the pD2 and maximum response comparison between groups with each other. Values were considered to be statistically significant at $P < 0.05$.

Results

In aortic segments from non-diabetic and diabetic rats, precontracted with endothelin-1(10−7 M) the addition of cumulative dosages of apelin (10^{-10}, $3*10^{-10}$, 10^{-9}, $3*10^{-9}$, 10^{-8}, $3*10^{-8}$, 10^{-7} $3*10^{-7}$ M) to the organ bath evoked concentration-dependent relaxation which was significantly lower in arteries from diabetic than in non-diabetic rats (Figure 1). The Emax value of the dose-response curve to apelin in diabetic rats was significantly lower (3.720± 0.5985 %) than that obtained in non-diabetic rats (22.43±2.828 %).

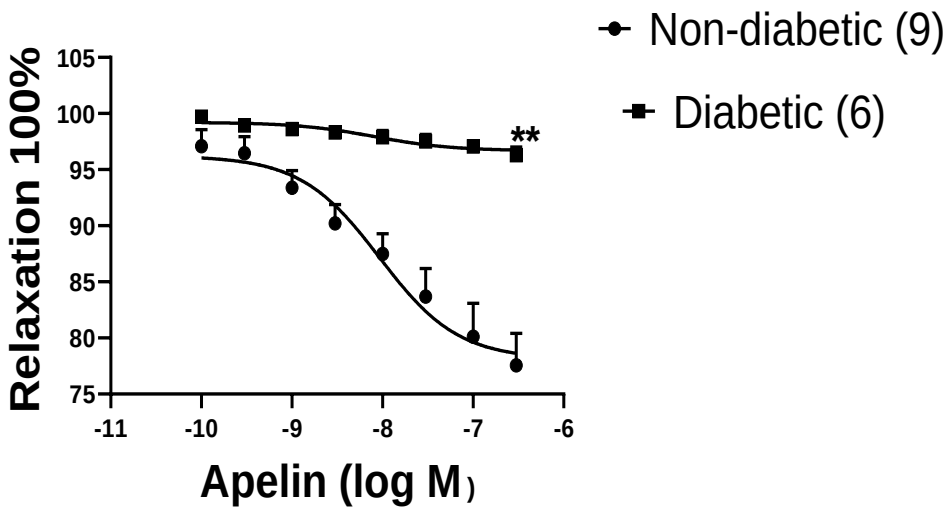

Figure.1: Concentration-response curves for apelin in endothelin-1 precontracted aortic artery isolated from non-diabetic and diabetic rats. * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

Table 1: The potency (pD2) and the maximum response (Emax) from the thoracic aortic rings responses to apelin in non-diabetic and STZ-induced diabetes

Groups	Non-diabetic			Induce Diabetes Militus		
	Number	**Emax (%)**	**pD_2**	**Number**	**Emax (%)**	**pD_2**
Control	9	22.43±2.828	-8.033±0.2079	6	3.720±0.5985	-8.035±0.3664 2.583 2.583
TEA	5	8.053±1.553****	-8.519±0.2348	4	8.250±1.659**	-8.475±0.3222
Chtx	5	9.819±2.580****	-8.033±0.2927	4	7.742±1.053*	-8.494±0.2866
Clotrimazole	5	4.788±0.7910***	-8.299±0.3046	4	0.9754±0.4088	-6.853±0.6543
$BaCl_2$	6	10.38±1.515****	-8.578±0.3316	4	3.755±0.1790	-7.538±0.1108
Glibenclamide	5	7.690±1.032****	-7.611±0.4884	4	2.219±0.6343	-7.142±0.4340
4-AP	5	9.164±1.551****	-8.398±0.2117	4	3.246±0.4394	-8.494±0.2866

The studied groups were compared with control group (ANOVA was applied with Dunnett-test). * Significant differences between the studied groups vs control group at P<0.001

The Effects of K_{ca} Channels on Aortic Response to Apelin in Non-diabetic and Diabetic Rats

After blocking the K_{ca} by TEA, the relaxant effect of apelin on the aortic segment reduced significantly, an event exhibited by the Emax reduction from 22.43±2.828% to 8.053±1.553% (Figure 2.A, Table 1). However, there was a slight elevation in relaxation altitude from 3.720±0.5985 to 8.250±1.659 in the diabetic group without significant change in potency. The highly significant alternation in vascular response to apelin in the diabetic group in presence of TEA is clearly represented in dUAC (Figure 2) indicating that the vasoactive effect of apelin strongly depends on K_{ca} channels which appear to be impaired in diabetes.

The efficacy of apelin in the subset group of non-diabetic rats increased from 22.43±2.828% to 9.819±2.580% by pre-incubation for 20 mints with chtx (1 µM), without significant change in potency. In contrast, STZ-induced diabetes completely reversed the maximum response from 3.720+0.5985 to 7.742+1.053, however apelin potency (pD2: -8.494 ± 0.2866) remained unchanged. The high significant change in the vascular response to apelin in diabetes condition which exhibited in dAUC, as shown in Figure 2B, reflects that diabetes-induced endothelium dysfunction disrupted the K_{ca}'s activities in the vascular response to apelin.

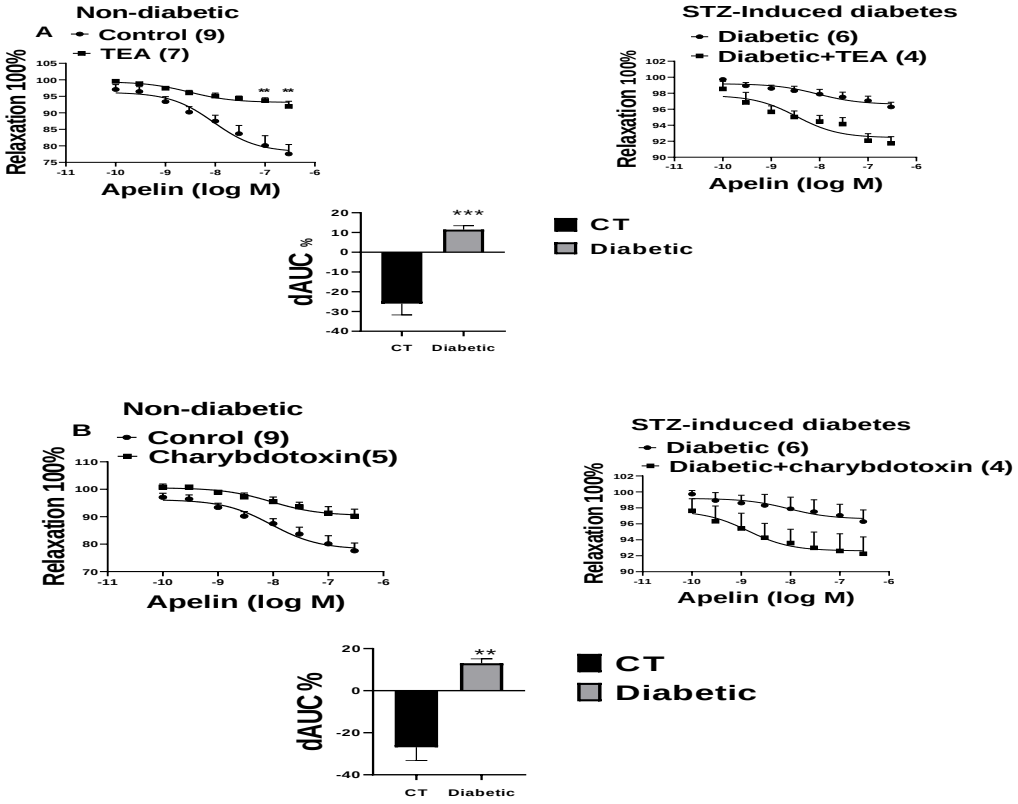

Figure 2: The effects of STZ-induced diabetes on the vasorelaxant responses to apelin in the thoracic aorta. (A) Effect of TEA 1 mM, and (B) Effect of chtx 1 µM on the vasorelaxant responses to apelin in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

Responses to clotrimazole, an IK_{ca} blocker, are shown in Figure 3A. The blocker abolished apelin relaxation and lowered the maximum response from 22.43±2.823% to 4.788±0.7910%.

In the diabetic group, exposure of rings to this blocker also decreased the maximum response to apelin from 22.43±2.828% to 4.788±0.7910% significantly (Table 1). In contrast no statistically significant changes were observed in apelin potency in both non-diabetic and diabetic groups. The dAUC showed a significant decrease in vascular reactivity to apelin in the presence of clotrimazole in diabetic aortic rings.

The effects of K_{ir} channel on vascular response to apelin in non-diabetic and diabetic rats

$BaCl_2$, a selective K_{ir} channel blocker was used to evaluate the role of the K_{ir} channels on vascular response to apelin. Barium chloride pre-incubation for 20 mins did not change the vascular relaxation response curve of apelin significantly (Figure 3B). Same effects were observed in diabetic vessels with statistically unchanged PD2 and Emax values (Table 1). On the other hand, marked effects of $BaCl_2$ in vascular responses to apelin in the non-diabetic group were clearly shown by dUAC, which remarkably diminished in diabetic states.

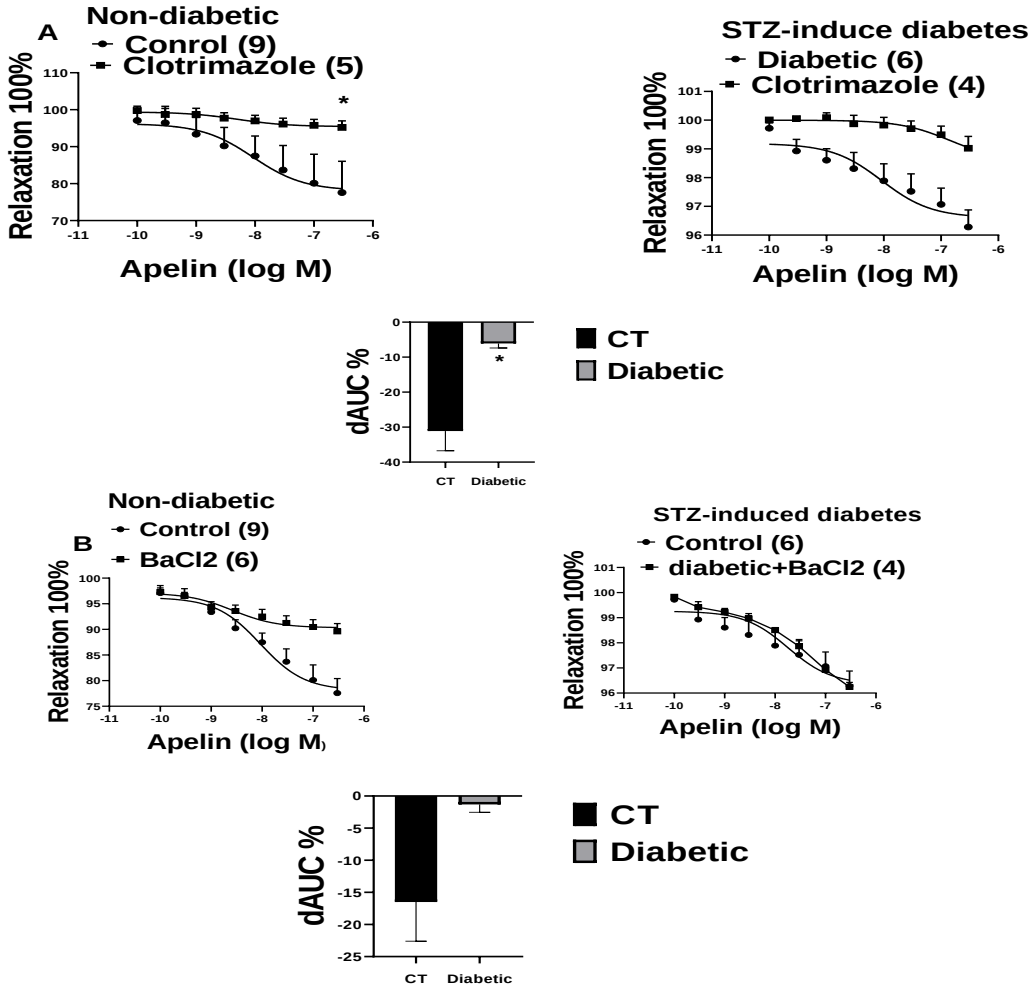

Figure 3: The effects of STZ-induced diabetes on the vasorelaxant responses to apelin in the thoracic aorta. (A) Effect of clotrimazole 10 µM, and (B) Effect of BaCL2 0.2 mM on the vasorelaxant responses to apelin in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

The Effects of K_v and K_{ATP} Channels on Aortic Response to Apelin in Non-diabetic and Diabetic Rats

Blocking K_v with 4-AP considerably moved the relaxation response curve to the right (Figure 4A), while it appeared to decrease the tissue's maximum relaxation response to apelin (Emax: 9.164±1.551%) when compared with control groups (Emax: 22.45±2.828%), with no significant change in the potency of 4-AP treated groups compared to control one (PD2: -8.398±0.2117 vs -8.033±0.2079) respectively as illustrated in Table 1, However, no significant increase in relaxation response was seen in diabetic arterial segments preincubated with 4-AP (Emax: 3.246±0.4394%) in comparison to control group (Emax: 3.720±0.5985%). The dAUC represented in Figure 4 demonstrates a high significant decrease in the relaxant effect of apelin in the diabetic group in the presence of 4-AP.

As it can be seen in Figure 4B, blocking of K_{ATP} channels by glibenclamide (30 µM) reduced maximum responses in non-diabetic l (Emax: 7.690 ± 1.032%). Whereas it did not affect the maximum response in the diabetic group (Emax:2.219 ± 0.6343). The dAUC showed abolishing effects of glibenclamide on the vascular responses to apelin with no significant change in the potency in non-diabetic and diabetic groups (Figure 4B and Table 1).

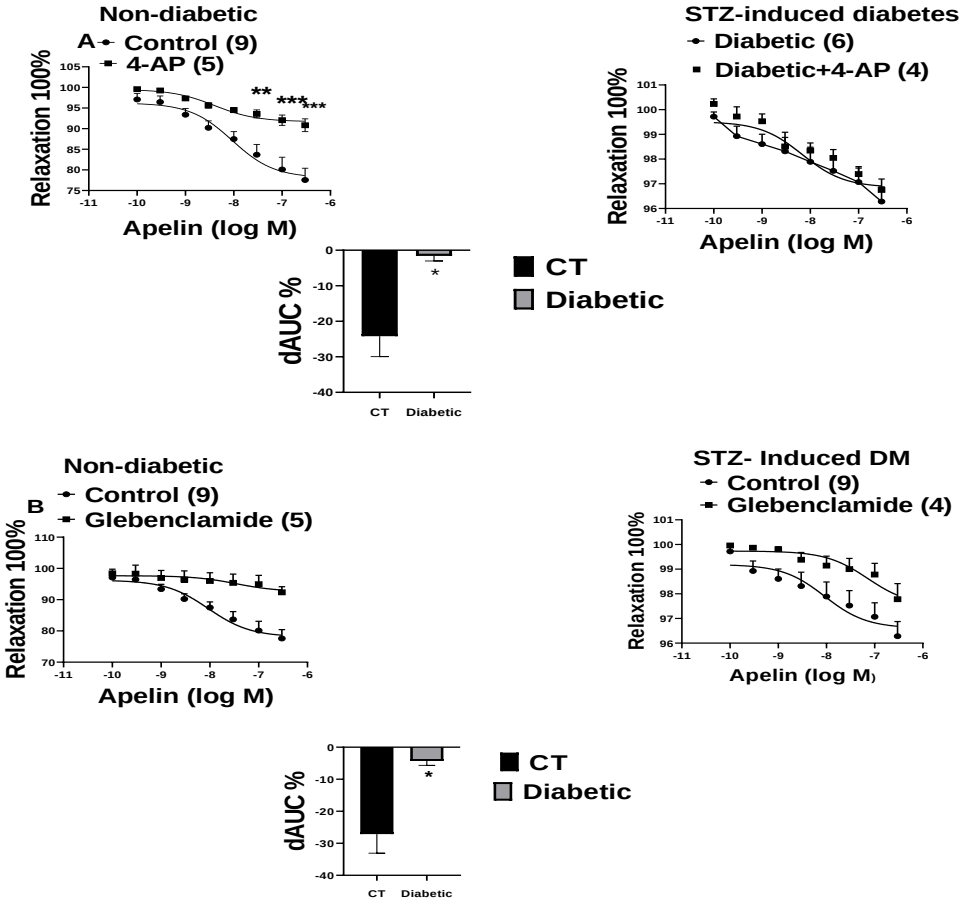

Figure 4: The effects of STZ-induced diabetes on the vasorelaxant responses to apelin in the thoracic aorta. (A) Effect of 4-AP 0.5 Mm, and (B) Effect of Glib 30 µM on the vasorelaxant responses to apelin in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

The Effects of EDRF on Vascular Responsiveness to Apelin in Non-diabetic and Diabetic Rats

Inhibiting aortic NOS with L-NAME moved the aortic response curve to the right and considerably reduced the efficacy of apelin-13 (Emax: 8.5561.4%) in comparison to non-diabetic aortic segments Emax: 22.43±2.828%. Whereas the response curve of the diabetic artery with the presence of the L-NAME, shifted to the left with no significant changes in both potency and efficacy (Figure 1A). The NO-dependent relaxant effect of apelin was further confirmed in dUAC which attenuated in diabetic conditions.

Incubation of aortic segments with the cyclooxygenase inhibitor indomethacin (10−5 M) potentiated the DRC of apelin in non-diabetic (Figure 5B) rats. In contrast Indomethacin in STZ-induced diabetic vessels moved the vascular response curve of apelin to the right and amplified the relaxation altitude significantly with (Emax: 12.34 ±2.447%) and caused no significant change in (PD2: -8.946±0.4087). The dAUC showed significant effects of indomethacin in the vascular responses to apelin in diabetic groups (Figure 5B).

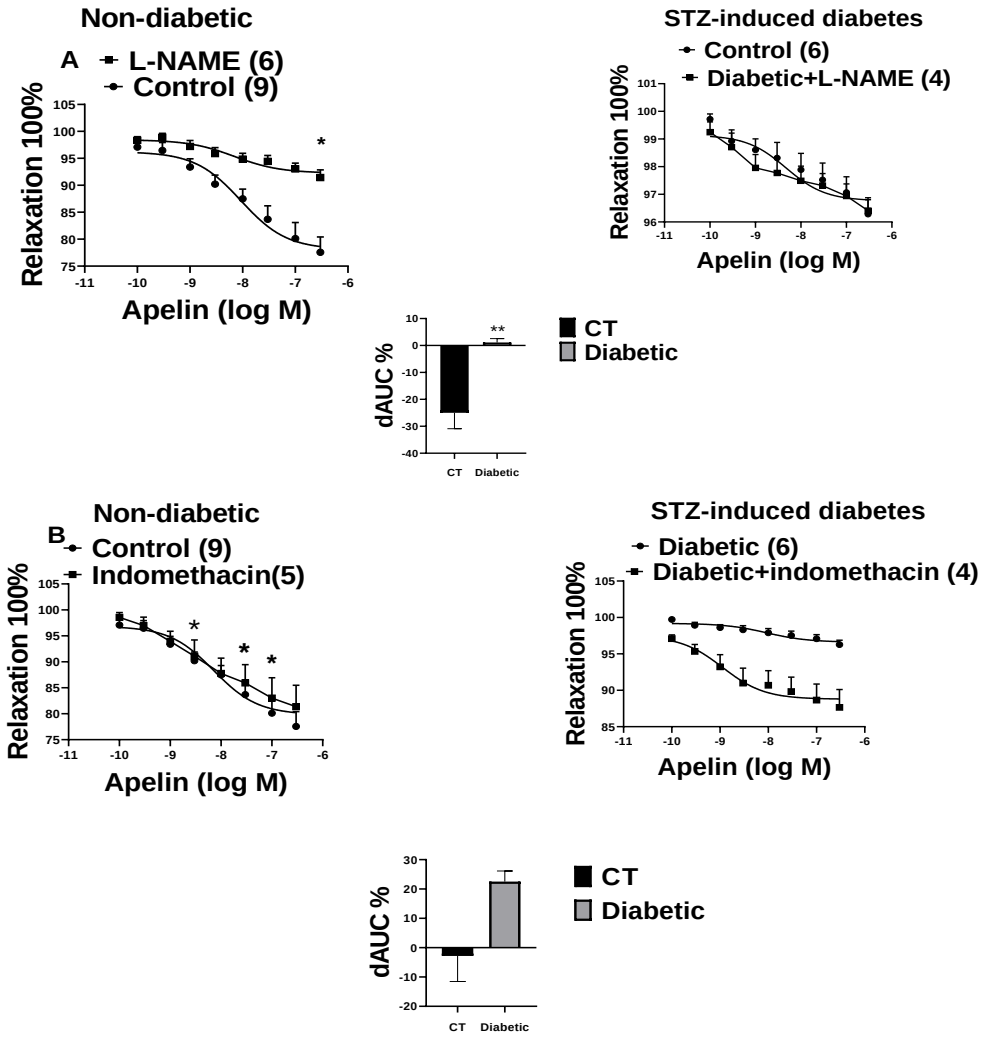

Figure. 5: The effects of STZ-induced diabetes on the vasorelaxant responses to apelin in the thoracic aorta. (A) Effect of L-NAME 200 µM, and (B) Effect of indomethacin mM on the vasorelaxant responses to apelin in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

Discussion

In the present study, for the first time we investigated, the influence of diabetes on the role of potassium channels and EDRF mediating the relaxant response of the rat aortic artery to apelin. Streptozotocin- induced diabetic group experienced a lower overall percentage of vasorelaxation than the non-diabetic group, where the peptide relaxed VSMC by 3.7% compared to 22.4% vasodilatation evoked by apelin in the non-diabetic group. Apelin elicited relaxation *via* two distinct routes one is L-NAME-sensitive and NO-mediated, while the other one is sensitive to TEA, clotrimazole, a K_{Ca} channel blocker and a 4-AP a K_v channel blocker. Based on the present results, diabetes-induced hypo-reactivity of the rat aorta to apelin and this effect is clearly evidenced by diminished potassium channel involvement and eliminated NOS activities. Since Zhong *et a.l* in a study observed that both mRNA and protein levels of the apelin receptor were lowered in the aortas of adult spontaneously diabetic mice [16], it is possible to hypothesize that limitation in relaxation response to apelin in STZ-induced diabetes in our study is a reflection of APJ receptor desensitization. Rapid desensitization of apelin-receptor has been demonstrated *in vitro* [2]. This desensitization of the response was obtained with respect to the inhibition of adenylyl cyclase and to phosphorylation of ERK and Akt [17]. Moreover, STZ-induced hypoglycemia in mice showed a reduction in Akt phosphorylation [18]. This could be a mechanism for the desensitization of the apelin receptor in the endothelial dysfunction induced by diabetes.

Despite their regulatory potentiation under normal conditions, apelin has been observed to involve in several organ pathophysiology [19] and plasma levels of apelin are up-regulated under pathological conditions such as vascular disease of coronary arteries [20], induced and diabetic patient [21, 22]. From this perspective, it is logical to speculate that the aortic artery hypo-reactivity to elevated plasma level of apelin reflects a greater compensatory attempt to prevent cardiovascular injury.

Regarding the contribution of potassium channels in apelin relaxant responses, alterations in vascular responsiveness were detected in diabetic aortic segments in compared to healthy groups. This research reported that inhibiting K_{Ca} channels with TEA lowered the vasorelaxation mediated by apelin, indicating that K_{ca} activation is accountable for the observed apelin vasodilatory effect which was consistent with [14] who discovered that apelin relaxes coronary arteries smooth muscle cells by enhancing K_{Ca} activity. It has been proven that K_{Ca} is a key regulator of the vascular tone and its activation causes a considerable increase in potassium efflux, resulting in hyperpolarization and subsequent dilation of VSMC [7].

The lowering in the amplitude of vascular response to apelin in diabetic aortic rings in the presence of TEA, on the other hand, provided valuable information, leading us to assume that downregulation or alternation of the K_{Ca} in diabetes is a mechanistic pathway of reduced vascular responses to apelin. Calcium activated potassium channel activities in VSMC have been observed to be altered in a variety of experimental models of diabetes [23]. Hyperglycemia results in β1-subunit down-regulation *via* activation of calcineurin/nuclear factor of activated T-cells, cytoplasmic 3 signaling that is facilitated by A-kinase anchoring protein 150 [24].

In non-diabetic rats, charybdotoxin, a scorpion venom toxin that is typically utilized in endothelial K_{Ca}-channel investigations, did not influence vascular reactivity to apelin, whereas diabetes reduced this reactivity. There are no reports until now explaining this mechanism. Diminished sensitivity of charybdotoxin-sensitive potassium channels, most likely BK_{Ca}, in the relaxing action of apelin in the diabetic artery, might be attributed, at least in part, by

hyperglycemia since BK_{Ca} beta1 subunit expression has been shown to be reduced in hyperglycemic condition [23].

Barium chloride, a K_{ir} channel inhibitor, was used to test the hypothesis that K_{ir} channels have participated in apelin-induced arterial relaxation. Inward rectifier potassium channels have been used as a pharmacological tool for evaluating the signal transduction pathways of various vasodilators because of their predominance in vascular endothelium and their importance in regulating vessel relaxation [25]. The present data does not support the participation of K_{ir} channels in the relaxation of rat aorta produced by apelin, because $BaCl_2$ failed to antagonize the arteries vasodilator response to apelin. On the other hand, a progressive decrease in vascular response to apelin in STZ- induced diabetic rats (Figure 3) supports the notion that diabetes alters the function of K_{ir} channels in the arteries of various experimental diabetes models.

Clotrimazole, a blocker of IK_{Ca} inhibited the arterial response to apelin in the non-diabetic group indicates the vital role of this channel in the relaxant response to apelin. While the vascular response to apelin in the diabetic group disappeared in the presence of this blocker, demonstrating that the decreased arterial response to apelin is linked to lesser participation of the above-mentioned potassium channel in diabetes. Our findings are consistent with those observed in coronary arteries, where diabetes has been shown to reduce SK_{Ca}/IK_{Ca} currents and limit SK_{Ca}/IK_{Ca} activator-mediated endothelium-dependent relaxation [26].

As clotrimazole has also been experimentally proven to inhibit cytochrome-P450 [27], an enzyme that converts arachidonic acid into powerful vasorelaxant products such epoxyeicosatrienoic acids [28]. With the use of this blocker, we were able to show for the first time that cytochrome P450-derived epoxyeicosatrienoic acids contribute to apelin-induced vasodilator tone in the rat aorta artery. Accordingly, our discovery that clotrimazole inhibited apelin-induced vascular responses was most likely due to cAMP-mediated activation or regulation of cytochrome P450 enzyme. Based on our results, diabetes reduced vascular sensitivity to the peptide in the presence of clotrimazole, demonstrating that endothelial dysfunction caused a reduction in p450 enzymatic activity. In experimentally induced diabetes, a decrease in p450 enzymatic activity has been observed [29].

The outcomes of this investigation demonstrate that 4-AP greatly reduces vasorelaxation induced by apelin peptide and, therefore, constitute the first evidence in rats to illustrate vascular hyperpolarization *via* K_v channel as a mechanism of apelin-induced relaxation. Adipocyte-derived vasorelaxants have been demonstrated to relax VSMC *via* stimulating Kv channels [30]. The exact mechanisms by which apelin caused vascular relaxation by opening Kv channels are still unknown, although it appeared to be mediated by APJ-induced cAMP activation following apelin binding to the APJ receptor, since cAMP-dependent activation of Kv channels in VSMC has been highlighted in studies by [31]. We wondered if diabetes modulates this pathway. Indeed, our results demonstrate significantly different magnitudes of suppression in the relaxation response to apelin reported in 4-AP preincubated diabetic rats. These unique findings together suggest that vascular hyperpolarization caused by k_v channel activation is the main mechanism of vasorelaxation in response to apelin and this pathway is impaired under diabetics. In type 1 diabetes experimental models, the expression and function of voltage-gated potassium channels have been observed to be restricted due to reactive oxygen species, K_v nitration and PKC activation [32].

The vasodilator efficacy of apelin considerably lowered in this study by the preincubation of glibenclamide, a drug that selectively inhibits vascular K_{ATP}-channels, and these declines were greater in diabetic than in control rats. This finding suggested the possibility that APJ

receptor activation by apelin is linked to the opening of these type of channels, that could contribute to peptide's relaxant effects. This result is related to the discovery that glibenclamide reduces utero relaxant effect responses to apelin in rat uterine horn [33]. Adenosine triphosphate sensitive potassium channels are unique in their ability to regulate membrane excitability and are involved in insulin production in beta cells of pancreas as well as insulin-dependent glucose absorption in skeletal muscle fibers [34]. The K_{ATP} activity has previously shown to be diminished in diabetes [35]. Glibenclamide is a drug derived from sulfonylurea that enhances insulin release by closing K_{ATP} (Sur1-Kir6.2) channels in pancreatic islet cells. It has played a vital role in the effective treatment of diabetes millitus [36]. The loss of apelin's' vascular beneficial effect due to K_{ATP} in the diabetic aorta with glibenclamide led us to hypothesize that this class of drug would have adverse vascular consequences in diabetic patients.

In the current study, we investigated whether the relaxing response of the aortic artery to apelin is mediated by NO. Accordingly, an irreversible NO inhibitor, L-NAME, which suppresses the eNOS enzyme, was used. It markedly attenuated the dilating effect of apelin, hence, revealing endothelial NO contribution in the relaxant effects of the apelin. Our findings are inconsistent with studies by Yu *et al*. [13] and Mughal *et al*. [37] who found that intravenous apelin administration decreased systolic and diastolic blood pressure in rats and this effect was suppressed by NOS inhibitors. Furthermore, a rat model study concluded that apelin-induced PI3K/Akt signaling increased phosphorylation of eNOS and modulated aortic vascular tone [38]. Our present study is the first time to elucidate the loss of vascular response to apelin in diabetic aortic rings preincubated with L-NAME. Diabetes may impair NO bioavailability due to hyperglycemia which limits activities and uncouples NOS due to low argininas levels [39].

In this observation, the involvement of cyclooxygenase-derived arachidonic acid metabolites in modulating the aorta's response to apelin, as well as the probable altering effects of diabetes, were investigated. There is insufficient data on the influence of prostanoids on the vasoactive action of apelin. In mammary arteries, various isoforms of apelin (apelin-13, -36, and [Pyr]-apelin-13) have been found to elicit relaxations which were suppressed when indomethacin was incubated [40]. Unexpectedly, the vasorelaxant effect of apelin was potentiated by indomethacin in this investigation, with potentiation being stronger in control than in diabetic rats. This strange result suggests that prostanoids alter the response of the rat aortic artery to apelin with a net vasoconstrictor impact which results in a decrease in diabetes. A possible mechanism to explain how indomethacin induces vasorelaxation is suggested by De-Angelis *et al* where they reported that indomethacin induces the release of reactive oxygen species and in particular of ONOO that (under physiological conditions) exerts sustained vasorelaxation through the production of compounds able to generate NO[41].

Conclusion

Our observations represent the first study to show that diabetes-induced endothelial impairment restricted the vascular response to apelin through impairment of potassium channel signaling and diminished NO activities. Understanding the underlying mechanisms of diabetes-induced reduction in vascular responses to apelin-13, as well as, the relation between many involved regulatory systems as EDRF and potassium channels may provide an opportunity to identify a new therapeutic option.

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Conflicts of Interest:

The authors declare that they have no conflicts of interest.

References

[1] D. Wu *et al.*, "Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer," (in eng), *Nature*, vol. 559, no. 7715, pp. 637-641, Jul 2018, doi: 10.1038/s41586-018-0350-5.

[2] Z. Zhu *et al.*, "HMGB1 impairs endothelium-dependent relaxation in diabetes through TLR4/eNOS pathway," (in eng), *Faseb j*, vol. 34, no. 6, pp. 8641-8652, Jun 2020, doi: 10.1096/fj.202000242R.

[3] S. Huang *et al.*, "Histone deacetylase 3 inhibition alleviates type 2 diabetes mellitus-induced endothelial dysfunction via Nrf2," (in eng), *Cell Commun Signal*, vol. 19, no. 1, p. 35, Mar 18 2021, doi: 10.1186/s12964-020-00681-z.

[4] K. Goto and T. Kitazono, "Endothelium-Dependent Hyperpolarization (EDH) in Diabetes: Mechanistic Insights and Therapeutic Implications," (in eng), *Int J Mol Sci*, vol. 20, no. 15, Jul 31 2019, doi: 10.3390/ijms20153737.

[5] C. R. Triggle, S. M. Samuel, S. Ravishankar, I. Marei, G. Arunachalam, and H. Ding, "The endothelium: influencing vascular smooth muscle in many ways," (in eng), *Can J Physiol Pharmacol*, vol. 90, no. 6, pp. 713-38, Jun 2012, doi: 10.1139/y2012-073.

[6] K. Schmidt and C. de Wit, "Endothelium-Derived Hyperpolarizing Factor and Myoendothelial Coupling: The in vivo Perspective," (in eng), *Front Physiol*, vol. 11, p. 602930, 2020, doi: 10.3389/fphys.2020.602930.

[7] W. F. Jackson, "Potassium Channels in Regulation of Vascular Smooth Muscle Contraction and Growth," (in eng), *Adv Pharmacol*, vol. 78, pp. 89-144, 2017, doi: 10.1016/bs.apha.2016.07.001.

[8] M. Kosmopoulos, D. Drekolias, P. D. Zavras, C. Piperi, and A. G. Papavassiliou, "Impact of advanced glycation end products (AGEs) signaling in coronary artery disease," (in eng), *Biochim Biophys Acta Mol Basis Dis*, vol. 1865, no. 3, pp. 611-619, Mar 1 2019, doi: 10.1016/j.bbadis.2019.01.006.

[9] N. Idris-Khodja *et al.*, "Endothelin-1 Overexpression Exaggerates Diabetes-Induced Endothelial Dysfunction by Altering Oxidative Stress," (in eng), *Am J Hypertens*, vol. 29, no. 11, pp. 1245-1251, Nov 1 2016, doi: 10.1093/ajh/hpw078.

[10] J. C. Zhong, Z. Z. Zhang, W. Wang, S. M. K. McKinnie, J. C. Vederas, and G. Y. Oudit, "Targeting the apelin pathway as a novel therapeutic approach for cardiovascular diseases," (in eng), *Biochim Biophys Acta Mol Basis Dis*, vol. 1863, no. 8, pp. 1942-1950, Aug 2017, doi: 10.1016/j.bbadis.2016.11.007.

[11] C. Read *et al.*, "International Union of Basic and Clinical Pharmacology. CVII. Structure and Pharmacology of the Apelin Receptor with a Recommendation that Elabela/Toddler Is a Second Endogenous Peptide Ligand," (in eng), *Pharmacol Rev*, vol. 71, no. 4, pp. 467-502, Oct 2019, doi: 10.1124/pr.119.017533.

[12] Y. X. Jia *et al.*, "Apelin activates L-arginine/nitric oxide synthase/nitric oxide pathway in rat aortas," (in eng), *Peptides*, vol. 28, no. 10, pp. 2023-9, Oct 2007, doi: 10.1016/j.peptides.2007.07.016.

[13] X. H. Yu *et al.*, "Apelin and its receptor APJ in cardiovascular diseases," (in eng), *Clin Chim Acta*, vol. 428, pp. 1-8, Jan 20 2014, doi: 10.1016/j.cca.2013.09.001.

[14] A. Mughal, C. Sun, and S. T. O'Rourke, "Activation of Large Conductance, Calcium-Activated Potassium Channels by Nitric Oxide Mediates Apelin-Induced Relaxation of Isolated Rat Coronary Arteries," (in eng), *J Pharmacol Exp Ther*, vol. 366, no. 2, pp. 265-273, Aug 2018, doi: 10.1124/jpet.118.248682.

[15] A. Modgil, L. Guo, S. T. O'Rourke, and C. Sun, "Apelin-13 inhibits large-conductance Ca2+-activated K+ channels in cerebral artery smooth muscle cells via a PI3-kinase dependent mechanism," (in eng), *PLoS One*, vol. 8, no. 12, p. e83051, 2013, doi: 10.1371/journal.pone.0083051.

[16] J. C. Zhong, X. Y. Yu, Y. Huang, L. M. Yung, C. W. Lau, and S. G. Lin, "Apelin modulates aortic vascular tone via endothelial nitric oxide synthase phosphorylation pathway in diabetic mice," (in eng), *Cardiovasc Res*, vol. 74, no. 3, pp. 388-95, Jun 1 2007, doi: 10.1016/j.cardiores.2007.02.002.

[17] B. Masri, N. Morin, L. Pedebernade, B. Knibiehler, and Y. Audigier, "The apelin receptor is coupled to Gi1 or Gi2 protein and is differentially desensitized by apelin fragments," *J Biol Chem*, vol. 281, no. 27, pp. 18317-26, Jul 7 2006, doi: 10.1074/jbc.M600606200.

[18] S. D. Robertson *et al.*, "Insulin reveals Akt signaling as a novel regulator of norepinephrine transporter trafficking and norepinephrine homeostasis," (in eng), *J Neurosci*, vol. 30, no. 34, pp. 11305-16, Aug 25 2010, doi: 10.1523/jneurosci.0126-10.2010.

[19] X. Sun, Y. Zhang, X. Qi, and L. Wei, "Impact of Apelin-13 on the Development of Coronary Artery Ectasia," (in eng), *Acta Cardiol Sin*, vol. 36, no. 3, pp. 216-222, May 2020, doi: 10.6515/acs.202005_36(3).20190901a.

[20] M. K. Akboga *et al.*, "Relationship between plasma apelin level and coronary collateral circulation," (in eng), *Atherosclerosis*, vol. 235, no. 2, pp. 289-94, Aug 2014, doi: 10.1016/j.atherosclerosis.2014.04.029.

[21] Y. Wang, R. Zhang, H. Shen, J. Kong, and X. Lv, "Pioglitazone protects blood vessels through inhibition of the apelin signaling pathway by promoting KLF4 expression in rat models of T2DM," (in eng), *Biosci Rep*, vol. 39, no. 12, Dec 20 2019, doi: 10.1042/bsr20190317.

[22] M. Habchi *et al.*, "Circulating apelin is increased in patients with type 1 or type 2 diabetes and is associated with better glycaemic control," (in eng), *Clin Endocrinol (Oxf)*, vol. 81, no. 5, pp. 696-701, Nov 2014, doi: 10.1111/cen.12404.

[23] M. A. Nystoriak *et al.*, "AKAP150 contributes to enhanced vascular tone by facilitating large-conductance Ca2+-activated K+ channel remodeling in hyperglycemia and diabetes mellitus," (in eng), *Circ Res*, vol. 114, no. 4, pp. 607-15, Feb 14 2014, doi: 10.1161/circresaha.114.302168.

[24] M. Nieves-Cintrón, V. A. Flores-Tamez, T. Le, M. M.-A. Baudel, and M. F. Navedo, "Cellular and molecular effects of hyperglycemia on ion channels in vascular smooth muscle," *Cellular and Molecular Life Sciences*, vol. 78, no. 1, pp. 31-61, 2021/01/01 2021, doi: 10.1007/s00018-020-03582-z.

[25] A. H. Ahmed and I. M. Maulood, "The roles of potassium channels in contractile response to urotensin-II in mercury chloride induced endothelial dysfunction in rat aorta," (in eng), *Iran J Vet Res*, vol. 19, no. 3, pp. 208-216, Summer 2018.

[26] Y. Liu *et al.*, "Inactivation of Endothelial Small/Intermediate Conductance of Calcium-Activated Potassium Channels Contributes to Coronary Arteriolar Dysfunction in Diabetic Patients," (in eng), *J Am Heart Assoc*, vol. 4, no. 8, p. e002062, Aug 24 2015, doi: 10.1161/jaha.115.002062.

[27] P. D. Crowley and H. C. Gallagher, "Clotrimazole as a pharmaceutical: past, present and future," (in eng), *J Appl Microbiol*, vol. 117, no. 3, pp. 611-7, Sep 2014, doi: 10.1111/jam.12554.

[28] J. Atone, K. Wagner, K. Hashimoto, and B. D. Hammock, "Cytochrome P450 derived epoxidized fatty acids as a therapeutic tool against neuroinflammatory diseases," (in eng), *Prostaglandins Other Lipid Mediat*, vol. 147, p. 106385, Apr 2020, doi: 10.1016/j.prostaglandins.2019.106385.

[29] M. Dostalek, M. H. Court, B. Yan, and F. Akhlaghi, "Significantly reduced cytochrome P450 3A4 expression and activity in liver from humans with diabetes mellitus," (in eng), *Br J Pharmacol*, vol. 163, no. 5, pp. 937-47, Jul 2011, doi: 10.1111/j.1476-5381.2011.01270.x.

[30] M. Gollasch, "Vasodilator signals from perivascular adipose tissue," (in eng), *Br J Pharmacol*, vol. 165, no. 3, pp. 633-42, Feb 2012, doi: 10.1111/j.1476-5381.2011.01430.x.

[31] J. B. Stott, V. Barrese, and I. A. Greenwood, "Kv7 Channel Activation Underpins EPAC-Dependent Relaxations of Rat Arteries," (in eng), *Arterioscler Thromb Vasc Biol*, vol. 36, no. 12, pp. 2404-2411, Dec 2016, doi: 10.1161/atvbaha.116.308517.

[32] M. Nieves-Cintrón, V. A. Flores-Tamez, T. Le, M. M. Baudel, and M. F. Navedo, "Cellular and molecular effects of hyperglycemia on ion channels in vascular smooth muscle," (in eng), *Cell Mol Life Sci*, vol. 78, no. 1, pp. 31-61, Jan 2021, doi: 10.1007/s00018-020-03582-z.

[33] "<ZUMJ_Volume 26_Issue 1_Pages 174-185.pdf>."

[34] T. P. Flagg, D. Enkvetchakul, J. C. Koster, and C. G. Nichols, "Muscle KATP channels: recent insights to energy sensing and myoprotection," (in eng), *Physiol Rev*, vol. 90, no. 3, pp. 799-829, Jul 2010, doi: 10.1152/physrev.00027.2009.

[35] S. S. Li *et al.*, "Impairment of the Vascular KATP Channel Imposes Fatal Susceptibility to Experimental Diabetes Due to Multi-Organ Injuries," (in eng), *J Cell Physiol*, vol. 230, no. 12, pp. 2915-26, Dec 2015, doi: 10.1002/jcp.25003.

[36] G. M. Martin, B. Kandasamy, F. DiMaio, C. Yoshioka, and S. L. Shyng, "Anti-diabetic drug binding site in a mammalian K(ATP) channel revealed by Cryo-EM," (in eng), *Elife*, vol. 6, Oct 24 2017, doi: 10.7554/eLife.31054.

[37] A. Mughal, C. Sun, and S. T. O'Rourke, "Apelin Does Not Impair Coronary Artery Relaxation Mediated by Nitric Oxide-Induced Activation of BK(Ca) Channels," (in eng), *Front Pharmacol*, vol. 12, p. 679005, 2021, doi: 10.3389/fphar.2021.679005.

[38] M. B. Wysocka, K. Pietraszek-Gremplewicz, and D. Nowak, "The Role of Apelin in Cardiovascular Diseases, Obesity and Cancer," (in eng), *Front Physiol*, vol. 9, p. 557, 2018, doi: 10.3389/fphys.2018.00557.

[39] A. Shatanawi, M. S. Momani, R. Al-Aqtash, M. H. Hamdan, and M. N. Gharaibeh, "L-Citrulline Supplementation Increases Plasma Nitric Oxide Levels and Reduces Arginase Activity in Patients With Type 2 Diabetes," (in eng), *Front Pharmacol*, vol. 11, p. 584669, 2020, doi: 10.3389/fphar.2020.584669.

[40] J. J. Maguire, M. J. Kleinz, S. L. Pitkin, and A. P. Davenport, "[Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease," (in eng), *Hypertension*, vol. 54, no. 3, pp. 598-604, Sep 2009, doi: 10.1161/hypertensionaha.109.134619.

[41] A. De Angelis, B. Rinaldi, A. Capuano, F. Rossi, and A. Filippelli, "Indomethacin potentiates acetylcholine-induced vasodilation by increasing free radical production," (in eng), *Br J Pharmacol*, vol. 142, no. 8, pp. 1233-40, Aug 2004, doi: 10.1038/sj.bjp.0705877.