ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Role of potassium channels in vascular contraction induced by chemerin in diabetic and non-diabetic isolated rat aorta

Hawzheen K.Othman*[1] , Almas MR. Mahmud[1] ,Ismail M. Maulood[1].
1Department of Biology, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq

A B S T R A C T:

tes can cause endothelial dysfunction and increase the risk of cardiovascular complications. Chemerin which has contractile effects on vascular smooth muscle cells. The purpose of the current investigation was to determine the possible involvement of potassium channels in the vascular response to chemerin and whether diabetes alters the processes behind the aortic vascular response to this peptide. In this study, rat thoracic aortic segments were pre-incubated with the chemerin, and potassium channels blockers including: non-selective calcium-activated potassium channel, big conductance calcium-activated potassium channels (BK_{ca}), intermediate conductance calciumactivated potassium channels (IK_{ca}), delayed inward rectifier potassium channels (K_{ir}), adenosine triphosphates-sensitive potassium channels (K_{ATP}) and voltage sensitive potassium channels (Kv) blockers, then cumulative concentrations of ET-1 were applied to each group in both non-diabetic and diabetic conditions. The non-selective K_{ca}, IK_{ca} blockers, slightly elevated ET-DRC but Kir, K_{ATP} and K_V blockers did have any effects on ET-1 DRC significantly. An important new finding in this study was that almost all potassium channel blockers noticeably $P<0.001$ decreased chemerin efficacy and increased pD2. The statistical analysis showed that endothelial damage caused by diabetes in the rat thoracic aorta significantly reduced the vascular responses to chemerin because, except for BaCl2, which potentiates ET-DRC and elevated Emax, all other blockers reduced ET-1DRC with a significant decrease of Emax. Importantly, diabetes increased the pD2 of chemerin when non-selective K_{ca}, BK_{ca}, and IK_{ca} blockers were pre-incubated, but the pD2 dramatically decreased when K_{ATP}, Kir, and K_V blockers were pre-incubated. In conclusion, K_{ca} channels contribute to the vasoconstrictive effects of chemerin. The enhanced vasoconstriction effects of chemerin are caused by impaired potassium channels in diabetes-induced endothelial dysfunction, which can be reduced by blocking these channels.

KEYWORDS: Chemerin, Diabetes, Endothelial dysfunction, Potassium channels Endothelin-1.
DOI: http://dx.doi.org/10.21271/ZJPAS.35.SpD.15
ZJPAS (2023) , 35(SpD);120-130

1.INTRODUCTION :

Diabetes is a metabolic disorder characterized by elevated blood glucose levels resulting from insufficient insulin secretion or action and is associated with an increased risk of cardiovascular complications, including atherosclerosis, hypertension, and endothelial dysfunction (Galicia-Garcia et al., 2020). The endothelium modulates vascular tone by producing vasoactive substances, such as nitric oxide, prostacyclin, and ET-1, which act on smooth muscle cells to regulate vascular diameter (Méndez-Barbero et al., 2021).

Endothelial dysfunction, which is characterized by impaired endothelium-dependent vasodilation, is an early marker of vascular damage in diabetes and plays a critical role in the development and progression of cardiovascular disease (Wang et al., 2022). Endothelial dysfunction in diabetes is associated with reduced production of nitric oxide and prostacyclin, leading to increased vasoconstriction and decreased vasodilation (Salvatore et al., 2021). Potassium channels, including BKca, Kv, and Kir, are critical in modulating vascular tone by regulating the membrane potential of smooth muscle cells (Daghbouche-Rubio et al., 2022).

Prolonged exposure to high glucose levels in mouse cerebral VSMC has resulted in a decrease

* **Corresponding Author:**
Hawzheen K.Othman
E-mail:
Article History:
Received: 09/04/2023
Accepted: 06/07/2023
Published:01/11/2023

in BKCa channel activity, which was attributed to the downregulation of the BKCa 1 subunit's activity(Lu & Lee, 2021). This downregulation may be caused by reduced protein cleavage or transcriptional expression of the BKCa 1 subunit during chronic hyperglycemia(Nieves-Cintrón et al., 2021). In addition, Various KV subunits' activities are altered by both acute and chronic hyperglycemia, leading to increased VSMC depolarization and vasoconstriction. PKC signaling is involved in acute glucose-mediated suppression of KV channels. However, the effect of diabetes on KIR channel function seems to be variable, possibly due to differences between regions, species, or models. It is also reasonable to assume that acute and short-term elevations in extracellular glucose may alter KATP channel activity, as hyperglycemia can alter the cellular metabolic status of vascular smooth muscle(Nieves-Cintrón et al., 2021). Chemerin, an 18 kDa protein, is known to regulate multiple biological processes including adipogenesis, glucose homeostasis, tumorigenesis, inflammation, angiogenesis, myogenesis, and immune cell migration (Macvanin et al., 2022). Recently, studies have also shown that chemerin can affect vascular tone through its interaction with specific receptors expressed on vascular smooth muscle cells (Ferland et al., 2020). A previous study concluded that chemerin increases the potency of phenylephrine and ET1-induced contraction via the Erk MAPK pathway(Lobato et al., 2012). An in-vitro investigation showed that chemerin reduces NO-dependent cGMP signaling, lowering vascular relaxation in the rat aorta, the author attributed this finding to enhanced free radical production following chemerin-induced endothelial NOS uncoupling (Neves et al., 2014). Further study revealed that the contraction of vascular smooth muscle induced by chemerin is highly dependent on various factors such as Gi proteins, Src, Rho kinase, and L-type calcium channels, which bring about changes in calcium flux. On the other hand, it is important to mention that Erk MAPK and PLC do not play a significant role in the modulation of calcium flux or the subsequent contraction of vascular smooth muscle triggered by chemerin (Ferland et al., 2017). Moreover, when the endothelium was removed, chemerin also effectively simulated chemerin receptor-dependent contraction of the rat aorta (Ferland et al., 2020).

Previous studies have indicated that induced T1DM patients have higher levels of chemerin in their serum (Sun et al., 2020; Elsehmawy et al., 2019). However, there is limited research on the effect of chemerin on vasomotion and how its elevated levels and endothelial dysfunction affect the contractile response. The study's primary objective is to examine the impact of potassium channels on the contraction response to chemerin in both non-diabetic and diabetic rat isolated aorta. The study aims to gain a better understanding of the mechanisms underlying diabetes-related vascular dysfunction, specifically related to chemerin and potassium channels.

2.Materials and methods

2.1 Animals

The present study utilized fifteen male albino rats with a mean weight of 225 ± 12.3 grams. The rats were housed in sterile plastic cages containing softwood chip bedding and maintained under standard animal house conditions (temperature of 23 ± 2 °C, relative humidity of 55 ± 10%, and about 12 hour light/dark cycles). They had free access to normal rodent food and tap water *ad libidum.* The experimental protocol was approved by the ethical and animal committees of the College of Science at Salahaddin University-Erbil. The research was conducted in the Biology department of the College of Science from March 2022 to August 2022.

2.2Induction of experimental diabetes

Rats were fasted for 6-12 hours and then induced with diabetes by a single intraperitoneal injection of STZ (40mg/kg) freshly dissolved in 10 mM citrate buffer, pH 4.5 (Yanni et al., 2021). Hyperglycemia was observed 48–72 hours after diabetes induction using a glucometer and represented as milligrams per deciliter. Diabetic rats with blood glucose levels of more than 300 mg/dL were selected for further experiments. Diabetic rats had blood glucose levels of more than 300 mg/Dl.After four weeks of diabetes induction, the diabetic rats were subjected to the experiments.

2.3Preparations of aortic rings and tension measurement

Aortic segments were separated from rats that had been anesthetized with a ketamine/xylazine combination (90 mg/kg, i.p. and 10 mg/kg, i.p., respectively) before being put in an icy-oxygenated Krebs bicarbonate solution comprising the following components in Mm.in Mm/L: 119 NaCl, 4.7 KCl, 1.2 KH2 PO4; 1.2

MgSO4, 1.5 CaCl2, 25 NaHCO3, and 11 glucose, pH = 7.4. Each aorta was cleansed carefully of adhering connective tissues and lipids while keeping endothelial integrity, then sliced into four rings (3mm in length). Each aorta segment was then attached between two stainless hooks and placed in an organ bath containing Krebs bicarbonate solution bubbled with a combination of roughly 95% O2 and 5% CO2.

The ring tension was manually adjusted to 2 g and the system was equilibrated for around 60 minutes, during that time the containers were washed with a new Krebs solution every 15 minutes. Following that, the vascular viability was tested using (KCl 60 µM), and the maximal contraction generated was used to determine the standard percentages of contractile responses. The aortic segments were cleaned multiple times and allowed to equilibrate for 30 minutes after maximal contraction reached a plateau.

2.4Experimental design

Changes in vessel tension were measured in chemerin preincubated and non-incubated aortic tissues in response to cumulative concentrations of ET1, added to the vessel bath in the presence of 20 min preincubation with the potassium channel blockers.

1- Non-diabetics

Group 1 control

Cumulative concentration-response relationships for the constriction effect of ET-1 (10−12, 5*10-11, 10−10, 5*10-10, 10−9, 5*10-9, 10−8 5*10-8 , 10−7 5*10-7, 10−6 M) (Spec-Chem Ind, China)

Group 2: Chemerin

The aortic segments were preincubated with chemerin alone and following chemerin (10-7M) then Cumulative concentration-response relationships for the constriction effect of ET-.

Group 3: TEA

A non-selective calcium-activated potassium channel blocker called TEA (1 mM) was first incubated in the aorta segments for 20 minutes, followed by the addition of the cumulative dosages of endothelin.

Group **4: Chemerin+ TEA**

A non-selective calcium-activated potassium channel blocker called TEA (1 mM) was first incubated for 20 inutes, followed by the addition of chemerin and the cumulative dosages of ET-1.

Group 5: Charybdotoxin

For 20 minutes, the aortic rings were treated with Chtx (1 M), a BKca and IKca channels blocker. ET-1 was then administered in cumulative dosages.

Group 6: Chemerin+ charybdotoxin

For 20 minutes, the aortic rings were treated with Chtx (1 M), a BKca and IKca channels blocker. Chemerin was then incubated, and finally, ET-1 was administered in cumulative dosages.

Group 7: Clotrimazole

Clotrimazole (30 M), a blocker of intermediate conductance calcium-activated potassium channels (IKca), was incubated with the aorta segments for 20 minutes before cumulative dosages of ET1 were introduced.

Group 8: Chemerin+clotrimazole

Clotrimazole (30 M), a blocker of intermediate conductance calcium-activated potassium channels (IKca), was incubated with the aorta segments for 20 minutes before chemerin was added and the cumulative dosages of ET1 were introduced.

Group 9: Barium chloride (BaCl2)

After applying a delay inward rectifier potassium channel (Kir) blocker called BaCl2 (0.2 mM) to the aorta segments for 20 minutes, cumulative dosages of ET1 were then performed.

Group 10: Chemerin+ BaCl2

After applying a delay inward rectifier potassium channel (Kir) blocker called BaCl2 (0.2 mM) to the aorta segments for 20 minutes, chemerin was added, and the cumulative dosages of ET1 were then performed.

Group 11: glibenclamide

The adenosine triphosphates-sensitive potassium channels (KATP) blocker glibenclamide (30 M) was applied to the aortic segments for 20 minutes. and the cumulative dosages of ET1 were subsequently provided.

Group 12: chemerin+ glibenclamide

The adenosine triphosphates-sensitive potassium channels (KATP) blocker glibenclamide (30 M) was applied to the aortic segments for 20 minutes. Chemerin was then incubated and the cumulative dosages of ET1 were subsequently provided.

Group 13: 4-aminopyridin (4-AP)

Voltage-sensitive potassium channels (KV) blocker 4-AP (0.5 mM) was applied to the aorta segments for 20 minutes, and then the cumulative dosages of ET1 were applied.

Group 14: chemerin+ 4-AP

Voltage-sensitive potassium channels (KV) blocker 4-AP (0.5 mM) was applied to the aorta segments for 20 minutes, and then chemerin was

applied, followed by the cumulative dosages of ET1.

2- **Streptozotocin induced diabetes**: The aorta segments of STZ-induced diabetic rats were also subjected to the groups mentioned.

2.5.Statistical analysis

The vascular contraction induced by chemerin was expressed as a percentage of tension generated by ET-1. To diferenrtiate between the effects of blockers in the aortic segments of non-diabetic and STZ-induced diabetic groups, all results were reported as differences in area under curves (dAUC). All data were expressed as means with standard error of the mean (SEM). We performed a two-way ANOVA to determine the differences between each dose of control and preincubated inhibitors, after which the Sidak post hoc test was applied to compare individual means. Furthermore, one-way ANOVA was used to compare the pD2 and efficacy among the studied groups followed by the Tukey multiple comparison test. The concentration or dose of medicine necessary to generate 50% of that drug's maximum action is known as pD2, sometimes referred to as pD2. While, efficacy or Emax is the maximal effect that may be predicted from this medicine. Values were considered to be statistically significant at $P < 0.05$.

3. Results

Endothelin-1 (10^{-12}–10^{-6}M) generated concentration-dependent contractions in the aortic arteries of non-diabetic rats with Emax of 80.02±4.97% and PD2 -9.164±0.096 respectively. Meanwhile, chemerin preincubation (10-7M) did not significantly alter the contraction response curve and efficacy, while it significantly lowered the pD2 -7.745±0.16) when compared with the pD2 of control -9.16±0.09. On the other hand, in diabetic state, a graphical representation of ET-1 concentration-dependent arterial contraction following pre-constriction with chemerin indicates that there was a slightly significant elevation in tension as shown in (Fig 1). Also, one-way ANOVA showed a significant rise in the chemerin group's pD2 (-8.9080.113), but no difference between the Emax of chemerin, which was 157.6 3.523%, and the control group, which was 157.217.14%. When compared to non-diabetes, the efficacy of the control (p-value 0.0001) and chemerin (p.value 0.0001) groups dramatically increased, but the efficacy of TEA and chemerin+TEA groups showed no significant difference. The effectiveness of the control group significantly declined (p>0.007) (Table 1).

The non-selective blocker of the Kca channels TEA increased the vasoconstriction response to ET-1 in chemerin pre-treated group significantly (Fig. 1). Under these conditions, the Emax of the TEA group increased significantly from 80.02±4.97% to 135.71± 19.8% . The TEA group's pD2 significantly dropped from (-9.16±0.09) to (-6.820.22) in that time. wereas the chemerin group pre-incubated with TEA had a considerable rise in pD2 (-10.890.341) when compared with the control-9.16±0.09 (Table 1, Fig 1) .

In diabetic states on the other hand, TEA alone and in combination with chemerin reduced ET-1-induced vasocontraction, the event was represented by significant reduction in EMAX of both (TEA) and (chemerin +TEA) 92.91 ± 2.85 and 71.75± 6.18 when compared with control 157.21±7.14. Also, a significant elevation of pD2 was found in TEA (-8.76±0.11) and chemerin+TEA, (-10.02±0.108) in comparison to control (-8.63±0.13), (Table 1 and Fig. 1).

Without a significant change in potency in the chemerin+tTEA group, that of chemerin and chemerin+TEA increased significantly with p values (0.0008) and (0.0002), respectively (Table 1).

3.1. The effects of BK_{ca} channels on aortic response to chemerin in non-diabetic and diabetic rats

The efficacy and contraction curves of Chtx and Chemerin+ Chtx decreased in the subset group of non-diabetic rats from 80.02±4.97% to (45.40 12.42%) and from 80.02±4.97% to (49.86 51.36%), respectively. Also the pD2 of the Chtx group significantly dropped (-7.4230.09), but the pD2 of the chemerin+Chtx group dramatically increased (-10.740.32) as compared to the control (-8.630.13). Moreover, STZ-induced diabetes significantly decreased the efficacy of Chtx and chemerin+Chtx, reducing the contraction curve from (157.21±7.14% to 61.69 1.43% and 157.21±7.14% to 20.47 3.75%, respectively). As a consequence, the pD2 of Chtx significantly decreased from -8.63±0.13 to -7.4230.09 while that of Chemerin+Chtx (pD2: -10.740.327) significantly elevated (Table 1 and Fig. 2). The

efficacy of chemerin+charybdotoxin was dramatically increased in diabetic rats (P>0.0001) compared to non-diabetic rats, however neither chemerin alone nor in combination with charybdotoxin significantly altered the Emax (Table 1).

3.2. The effects of IK_{ca} channels on aortic response to chemerin in non-diabetic and diabetic rats

While neither pre-incubation with clotrimazole nor the combination of chemerin and clotrimazole significantly changed the ET-I DRC or Emax, their pD2 considerably increased(-9.78± 0.09 and9.92± 0.21, respectively). (Table 1, Fig. 3). On the other hand, clotrimazole and a combination of chemerin and clotrimazole pre-incubation resulted in significant downward curve shifts in the diabetes groups.

This event was demonstrated by a significant decrease in the efficacy of both clotrimazole and clotrimazole + chemerin (66.18± 6.85%), (66..81± 7.91%), when compared to the Emax of the control group (157.21± 7.14%). Yet, the pD2 dramatically rose from -8.63± 80.13 to -9.480.27in clotrimazole and to -8.97±0.138 in the chemerin+clotrimazole group (Table 1, Fig.3). Chemerin alone or in combination with clotrimazole did not significantly change the Emax, however the efficacy of chemerin+clotrimazole was significantly reduced in diabetic rats (p>0.0157)compared to non-diabetic rats (Table 1).

3.3 Effect of K_{Ir} channels on aortic response to chemerin in non-diabetic and diabetic rats

The findings showed that pre-incubating BcCl2 with non-diabetic rat aortic rings did not significantly change the contraction curve or Emax of any of the groups examined, but the pD2 of the BaC2 group was significantly lower (-8.01± 0.21) than that of the chemerin group (-9.16±0.09). However, the contraction curve was shifted to the right in diabetic arterial segments that had previously been pre-incubated with BaCl2 (Emax: 41.64± 8.63), whereas the curve was potentiated (Emax: 176.4 ± 32.24%) by chemerin and BaCl2. In addition, when compared to the pD2 of control (-8.63± 0.13), the potencies of BaCl2 (-8.56±0.32) and chemerin+BaCl2 (-8.062±0.195) also significantly declined (Table 1 and Fig 4).

T-test analysis demonstrates the marked decrease in Emax of BaCl2 (p>0.021) and a notable ncrease in chemerin+BaCl2 (p>0.016) in diabetics with no appreciable change in their pD2 (Table 1).

3.4. The effects of K_{cATP} channels on aortic response to chemerin in non-diabetic and diabetic rats

The ET-1 concentration-response curve was somewhat lowered by the K_{ATP} channel blocker glibenclamide, while the chemerin group did not show any significant changes. Although hemerin efficacy remained largely unchanged at 61.02± 3.89 and 93.42 7.42 % in both glibenclamide and chemerin+ glebenclamide respectively, the pD2 levels of the glibenclamide demonstrated a significant rise (-9.04±0.121), however, that of chemerin+glebinclamide (-7.59± 0.12) decreased significantly when compared with control (-8.63 ± 0.13).

While chemerin efficacy was largely unchanged in both glibenclamide and chemerin+ glebenclamide at 61.02 3.89 and 93.42 7.42%, respectively, the pD2 levels of glibenclamide showed a significant increase (-9.040.121) incontrast those of chemerin+ glebenclamide (-7.59 0.12) decreased significantly when compared with control (-8.63 0.13). Moreover, the contraction altitude was significantly shifted downward in the glibenclamide and chemerin+glibenclamide groups, with a corresponding drop in efficacy of (157.63.523% to 36.917.71%) and (157.63.523% to 65.514.46), but no appreciable changes in pD2 (Table 1 and Fig. 5). Glebinclamide alone decreased efficacy (0.007) with no significant change in potency. When combined with chemerin, glibenclamide also lowered efficacy (p>0.036) while dramatically increasing pD2 (p>0.007) (Table 1).

3.5The effects of KV channels on aortic response to chemerin in non-diabetic and diabetic rats

The results demonstrated that pre-incubating non-diabetic rat aortic rings with 4-AP considerably potentiated the ET-1DRC, but that the combination of chemerin and 4-AP did not significantly change the contraction curve on and Emax () when compared to the control (80.02±4.97). The 4-AP's PD2 increases in comparison to the control group's, but the chemerin +4-AP's PD2 dramatically decreases. (Table 1 and Fig. 6). Conversely, diabetic arterial segments pre-incubated with BaCl2 and chemerin+BaCl2 altered the contraction curve to the right, which can be observed in the lowering of Emax to 62.38 3.3 and97.2910.49, respectively,

in comparison to chemerin's Emax (157.6 3.523) (Table 1 and Fig.6). Although the 4-AP group's efficacy dramatically decreased (p> 0.038) and its pD2 significantly increased (p> 0.007) in diabetics, the addition of chemerin did not affect any of these factors (Table 1).

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

	Emax %			pD2		
	Non-diabetes	*Induced-T1DM*	P values	*Non-diabetes*	*Induced-TIDM*	P values
Control	80.02±4.97	157.21±7.14	0.0001	-9.16±0.09	-8.63±0.13	0.0071
Chemerin	96.71±6.06	157.6± 3.523	0.0001	-7.745±0.16***	-8.908±0.11**	0.0008
TEA	135.7± 19.8***	92.91±2.85****	NS	-6.82±0.22***	-8.76±0.11***	0.0002
Chemerin+TEA	95.21± 12.86	71.75±6.18***	NS	-10.89±0.341***	-10.02±0.10**	NS
Chtx	45.4±12.4**	61.6± 1.4***	NS	-7.364± 0.79***	-7.42±0.09***	NS
Chemerin+chtx	49.8±51.3**	20.4± 3.7***	NS	-7.86± 0.07***	-10.74±0.32**	0.0001
Clotrimazole	69.72 ± 3.37	66.18± 6.8**	NS	-9.78±0.09**	-9.48±0.27***	NS
Chemerin+clotrimazole	96.04± 8.83	66.81± 7.9**	NS	-9.92±0.21****	-8.97±0.138**	0.0157
BaCl2	78.72± 8.32	41.64± 8.6**	0.021	-9.02±0.18	-8.56±0.3***	NS
Chemerin+BaCl2	92.32±14.06	176.4±32.2**	0.016	-8.013±0.21***	-8.06±0.19****	NS
Glibenclamide	61.02 ± 3.89	36.91± 7.7**	0.0074	-9.04±0.16	-9.04±0.12***	NS
Chemerin+Glebinclamide	93.421±7.46	65..5± 4.4***	0.036	-8.47±0.17***	-7.5±0.12***	0.0079
4-AP	102.1±16.5*	62.38± 3.3**	0.038	-6.88±0.26****	-9.76±0.16***	0.0001
Chemerin+4AP	86.18± 6.43	97.2±10.4***	NS	-8.23±0.209****	-7.7±0.13***	NS

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

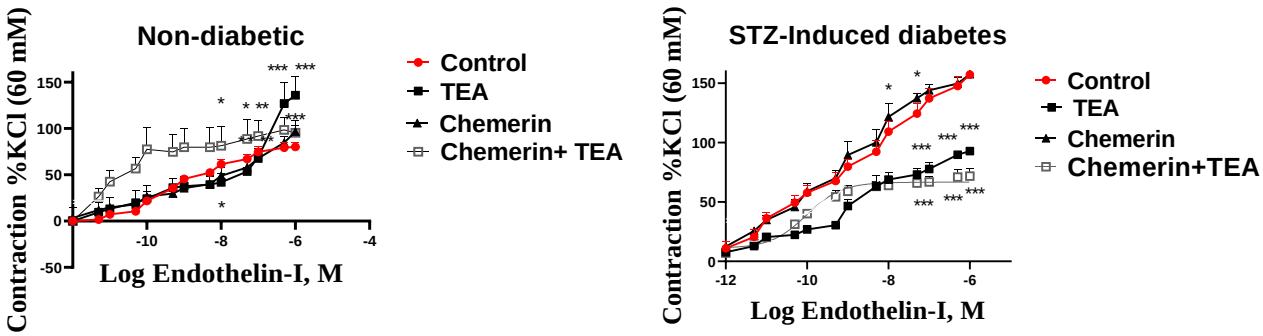

Fig. 1: Effect of TEA 1 mM, on the vasoconstriction responses to chemerin and ET-1 in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

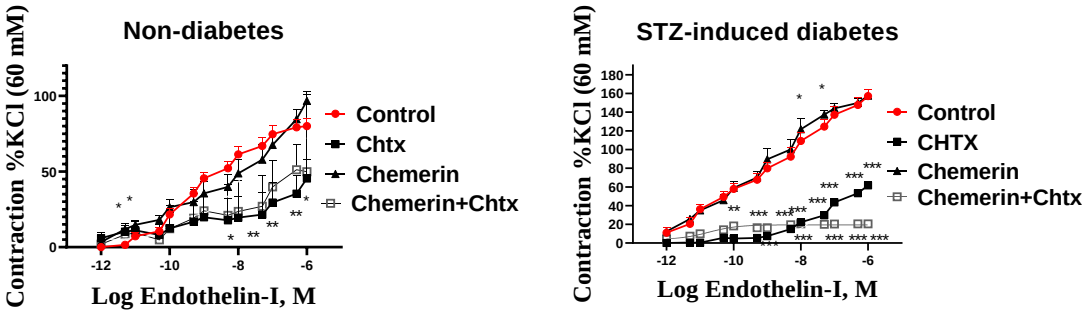

Fig. 2: Effect of charybdotoxin 1 µM, on the vasoconstriction responses to chemerin and ET-1 in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

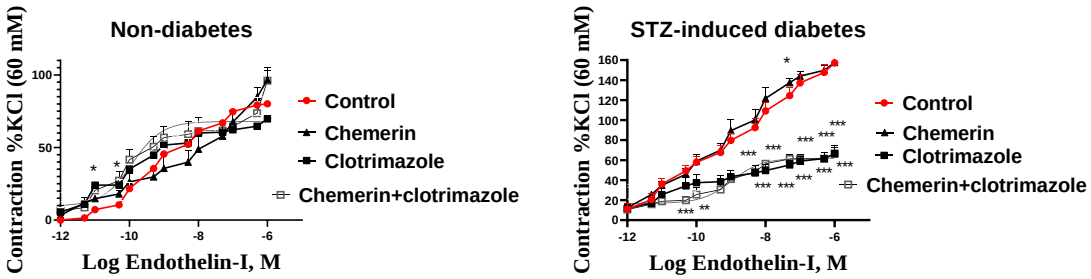

Fig. 3: Effect of clotrimazole 10 µM, on the vasoconstriction responses to chemerin and ET-1 in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

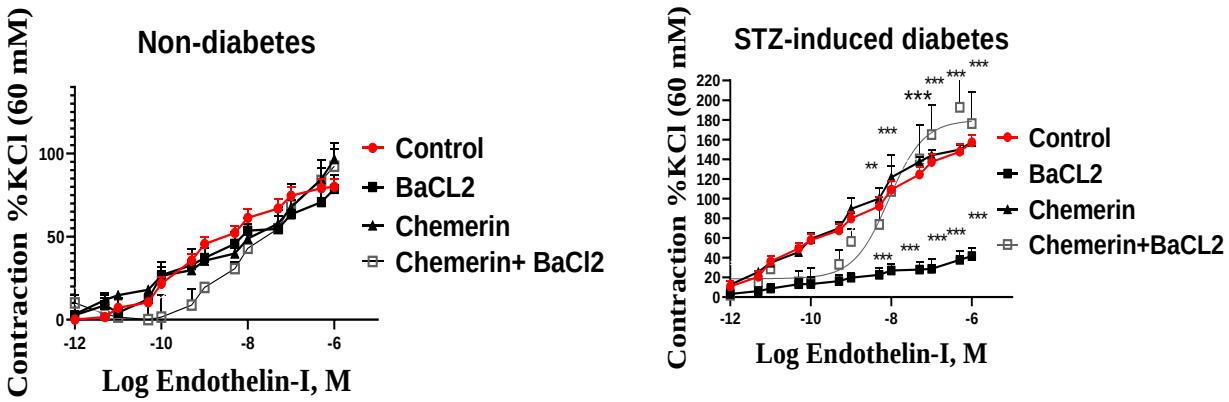

Fig. 4: Effect of BaCl2 0.2 mM, on the vasoconstriction responses to chemerin and ET-1 in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

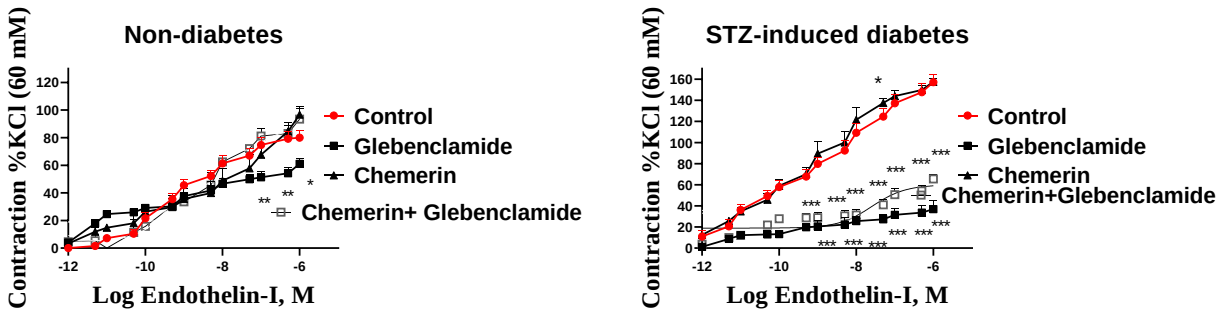

Fig. 5: Effect of Glibenclamide 30 µM, on the vasoconstriction responses to chemerin and ET-1 in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

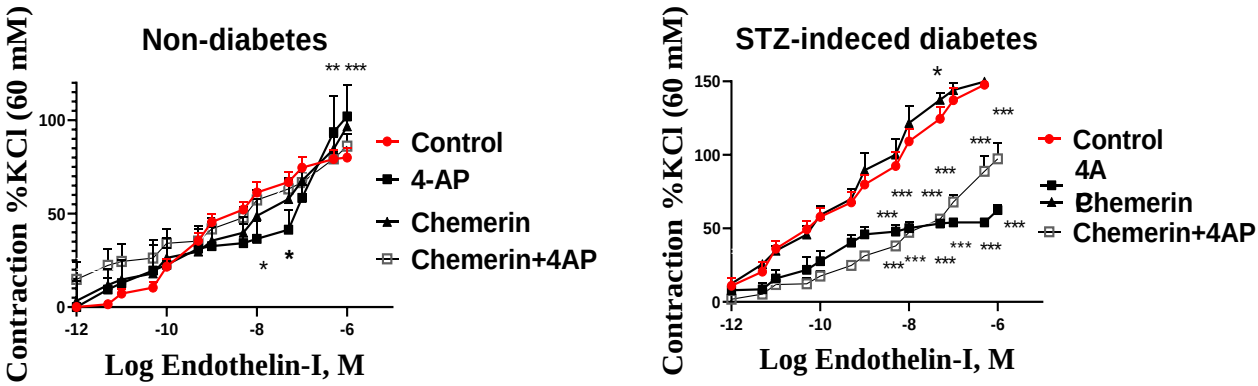

Fig. 6: Effect of 4-AP 0.5 Mm, on the vasoconstriction responses to chemerin and ET-1 in non-diabetic and diabetic aortic segments. The inset graphs show differences in area under the concentration-response curve (dAUC). * Represents statistical differences at P<0.05, and *** Represents statistical differences at P<0.001 versus the control group. The number of animals used is indicated in parentheses.

Discussion

This study is the first to investigate the pharmacological properties of chemerin, an exogenous vasoconstrictor, on isolated aorta arteries from non-diabetic and diabetic rats. The study also examined the blocking of potassium channels on the vascular effects of chemerin. The findings of the study indicate that chemerin has a greater contractile effect on diabetic arteries compared to non-diabetic arteries, particularly when precontracted with ET-1. Additionally, the study showed that efficacy of the chemerin groups increased significantly in diabetics.

There could be various mechanisms responsible for the increased vasoconstriction effects of chemerin in diabetic aortic arteries, such as greater availability of chemerin at the ETA receptor level or increased susceptibility of blood vessels to chemerin's vasoconstrictor effects. Our perviuos still unpublished study found that diabetic patients had elevated levels of chemerin in their serum, which supports the hypothesis of a positive association between chemerin and increased vasoconstriction in diabetes. Studies have also demonstrated that chemerin enhances the production of mitochondrial ROS when applied to human aortic vascular endothelium(Shen et al., 2013). Additionally, perivascular adipose tissue can produce various adipose tissue-derived constricting factors, leading to vasoconstriction and activation of the NADPH oxidase enzyme, which boosts reactive oxygen species production (El husseny et al., 2017). When chemerin increases the potency and efficacy in diabetes, it means that it is more effective at inducing its physiological response and that it requires a lower concentration or dose to produce the same maximal effect compared to non-diabetic conditions. The mechanism by which diabetes increases the potency and efficacy of chemerin-induced contraction is not fully understood, but it

may be related to the dysregulation of various signaling pathways and cellular processes in diabetes. For example, hyperglycemia and insulin resistance can lead to oxidative stress, inflammation, and endothelial dysfunction, These pathways may enhance the sensitivity of vascular smooth muscle cells to chemerin, resulting in increased potency and efficacy of chemerin-induced contraction.

The potentiation of the chemerin preincubated ET-1 concentration-response curve after TEA pretreatment suggests that the potassium channels that are blocked by TEA may be involved in counteracting the vasoconstrictive effects of chemerin. It is also possible that chemerin may activate potassium channels, such as Kca channels, that can counteract the vasoconstrictive effects of ET-1. The vasoconstrictive effects of ET-1 can lead to an increase in intracellular calcium levels in smooth muscle cells, which can activate Kca . Vasoconstrictors have been demonstrated to cause Kca channels to open up in response, acting as a counterbalance to their constriction-inducing effects(Jackson, 2017). The activation of KCa channels leads to hyperpolarization of the VSMC membrane potential, which reduces calcium entry into the cells and ultimately leads to the relaxation of VSMC (Hu & Zhang, 2023). However, in diabetic states, it appears that the effects of TEA on the chemerin-preincubated ET-1 concentration-response curve are reversed. Endothelial dysfunction in diabetes may affect the expression and function of these potassium channels, leading to a different response to TEA compared to non-diabetic conditions.

Although blocking BK_{ca} channels with charybdotoxin did not affect the vascular response to chemerin, diabetes-induced endothelial dysfunction reduced the sensitivity of blood vessels to the peptide. This suggests that endothelial dysfunction can alter vascular behavior and impair the response to vasoactive agents, particularly by affecting potassium channels. Charybdotoxin may decrease aortic constriction induced by chemerin and ET-1 by modulating the signaling pathways activated by these molecules. This could involve blocking the chemerin or ET-1 receptor on vascular smooth muscle cells, preventing downstream signaling, and reducing constriction.

The findings of this study suggest that clotrimazole has a modulatory effect on the vasoconstrictor response to ET-1 in both normal and diabetic aortic rings pre-incubated with chemerin. In the context of vasoconstriction effects of vasoconstrictors, clotrimazole can increase these effects by further reducing the counteracting vasodilatory response mediated by KCa channels. With KCa channels blocked by clotrimazole, the depolarizing effects of vasoconstrictors, such as ET-1, are less effectively counteracted, leading to increased vasoconstriction and vascular tone The observed differences in the effects of clotrimazole between normal and diabetic aortic rings suggest that diabetes may alter the function of Kca channels ().

The present study investigated the effects of BaCl2, a Kir potassium channel blocker, on the vasoconstriction response to ET-1 and chemerin in ET-DRC (ET-1 pre-contracted aortic rings). Our results showed that BaCl2 did not significantly alter the contraction response to ET-1 in ET-DRC, suggesting that KIR channels may not play a significant role in ET-1-induced vasoconstriction in this model On the other hand, BaCl2 potentiated the vasoconstriction response to chemerin in ET-DRC in diabetics, one possible mechanism for this effect is that chemerin may have the ability to inhibit Kir channels. Kir has been previously investigated to be inhibited by vasoconstrictors through activating signaling pathways involving PKC and tyrosine kinase, which can lead to the closure of Kir channels and subsequent depolarization of smooth muscle cells. This depolarization can lead to an influx of calcium ions and subsequent contraction of the blood vessel (Zitron et al., 2004). By inhibiting Kir channels, chemerin may amplify the depolarizing effects of vasoconstrictors like endothelin and further exacerbate the vasoconstrictor response in diabetes. It is worth noting that the interaction between BaCl2, chemerin, and Kir channels is complex and can vary depending on the specific context. Further research is needed to fully understand the mechanisms underlying these effects and their implications for cardiovascular health and disease. If BaCl2 is shown to increase the efficacy of chemerin-induced contraction in diabetics, it suggests that Kir channels might play a role in modulating the contractile response to chemerin. Specifically, the inhibition of Kir channels by BaCl2 might increase the depolarization and calcium influx in smooth muscle cells, leading to stronger and more prolonged contraction

Glibenclamide has been shown to decrease vascular contraction induced by both ET-1 DRC

and chemerin pre-incubated ET-1 DRC. The results of this study suggest that glibenclamide has KATP channel-independent effects on the rat isolated aorta, The muscle relaxant effect of glibenclamide appears to be a separate property from its role as a selective blocker of KATP channels. Blocking KATP channels would typically lead to membrane depolarization of smooth muscle cells, which would promote Ca2+ influx and increase vascular tension. However, studies have found that glibenclamide has a potent inhibitory effect on thromboxane A2-induced contraction in mammalian arteries and can also inhibit contractile responses to various constrictors such as phenylephrine, endothelin-I, and high K+ (Stanke et al., 1998). Glebiclamide has also been shown to induce both endothelium-dependent and -independent relaxation. The endothelium-dependent relaxation is primarily mediated by NO, while the endothelium-independent relaxation is likely due to interference with Ca2+ influx through voltage-sensitive Ca2+ channels or the protein kinase C-mediated contractile mechanism (Chan et al., 2000). The exact mechanism of how KATP channels interact with chemerin signaling pathways in diabetes is not fully understood and might depend on the specific tissue and experimental conditions. However, one possibility is that KATP channels might be dysregulated in diabetic smooth muscle cells, leading to altered membrane potential and ion channel expression. By inhibiting KATP channels, glibenclamide could restore the normal membrane potential and reduce the calcium influx, thus attenuating the downstream signaling events of chemerin.

Although blocking of Kv channels with 4-AP did not affect the vascular response to chemerin, the decreased vascular sensitivity to chemerin observed in diabetes suggests that endothelial dysfunction can alter vascular behavior and diminish the response to vasoactive agents. This pattern of signaling mechanisms is not unique, as demonstrated by similar findings with 4-AP, which can induce relaxation of arteries pre-contracted with noradrenaline through activation of Kv7.4 channels, direct or indirect activation of BKCa channels, and attenuation of noradrenaline signaling, partly dependent on intracellular pH (Khammy et al., 2018). Furthermore, earlier research has indicated that 4-AP has the ability to penetrate the cell membrane and bind with intracellular protons, resulting in the creation of 4-APH+ and an increase in intracellular alkalization. This process ultimately facilitates and enhances the relaxation rate (Choquet & Korn, 1992).

Conclusion

Calcium-activated potassium channels play a crucial role in regulating the vasoconstrictive effects of chemerin in vascular smooth muscle cells by hyperpolarizing the cell membrane and promoting relaxation of the vessel. However, in diabetes-induced endothelial dysfunction, the activity of all potassium channels may be impaired, leading to increased constriction response to chemerin. By blocking potassium channels, the vasoconstrictive effects of chemerin are reduced, which may have a positive impact on endothelial dysfunction produced by diabetes.

Acknowledgment: I appreciate Dr.Almas and Dr.Ismail's participation in examining early drafts of the full work critically.

Conflict of interest

I do not have any conflict of interest or any other relevant connection or shared interest.

References

Chan, W.-K., Yao, X., Ko, W.-H., & Huang, Y. (2000). Nitric oxide mediated endothelium-dependent relaxation induced by glibenclamide in rat isolated aorta. *Cardiovascular Research*, *46*(1), 180-187. https://doi.org/10.1016/s0008-6363(99)00423-x

Choquet, D., & Korn, H. (1992). Mechanism of 4-aminopyridine action on voltage-gated potassium channels in lymphocytes. *The Journal of general physiology*, *99*(2), 217-240.

Daghbouche-Rubio, N., López-López, J. R., Pérez-García, M. T., & Cidad, P. (2022). Vascular smooth muscle ion channels in essential hypertension. *Front Physiol*, *13*, 1016175. https://doi.org/10.3389/fphys.2022.1016175

El husseny, M. W. A., Mamdouh, M., Shaban, S., Ibrahim Abushouk, A., Zaki, M. M. M., Ahmed, O. M., & Abdel-Daim, M. M. (2017). Adipokines: Potential Therapeutic Targets for Vascular Dysfunction in Type II Diabetes Mellitus and Obesity. *Journal of Diabetes Research*, *2017*, 8095926. https://doi.org/10.1155/2017/8095926

Ferland, D. J., Darios, E. S., Neubig, R. R., Sjogren, B., Truong, N., Torres, R., Dexheimer, T. S., Thompson, J. M., & Watts, S. W. (2017). Chemerin-induced arterial contraction is G(i)- and calcium-dependent. *Vascul Pharmacol*, *88*, 30-41. https://doi.org/10.1016/j.vph.2016.11.009

Ferland, D. J., Mullick, A. E., & Watts, S. W. (2020). Chemerin as a Driver of Hypertension: A Consideration. *American Journal of Hypertension*, *33*(11), 975-986. https://doi.org/10.1093/ajh/hpaa084

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of Type 2

Diabetes Mellitus. *Int J Mol Sci*, *21*(17). https://doi.org/10.3390/ijms21176275

Hu, X.-Q., & Zhang, L. (2023). Ca2+-Activated K+ Channels and the Regulation of the Uteroplacental Circulation. *International Journal of Molecular Sciences*, *24*(2), 1349. https://www.mdpi.com/1422-0067/24/2/1349

Jackson, W. F. (2017). Potassium Channels in Regulation of Vascular Smooth Muscle Contraction and Growth. *Adv Pharmacol*, *78*, 89-144. https://doi.org/10.1016/bs.apha.2016.07.001

Khammy, M. M., Kim, S., Bentzen, B. H., Lee, S., Choi, I., Aalkjaer, C., & Jepps, T. A. (2018). 4-Aminopyridine: a pan voltage-gated potassium channel inhibitor that enhances K(v) 7.4 currents and inhibits noradrenaline-mediated contraction of rat mesenteric small arteries. *Br J Pharmacol*, *175*(3), 501-516. https://doi.org/10.1111/bph.14097

Lobato, N. S., Neves, K. B., Filgueira, F. P., Fortes, Z. B., Carvalho, M. H., Webb, R. C., Oliveira, A. M., & Tostes, R. C. (2012). The adipokine chemerin augments vascular reactivity to contractile stimuli via activation of the MEK-ERK1/2 pathway. *Life Sci*, *91*(13-14), 600-606. https://doi.org/10.1016/j.lfs.2012.04.013

Lu, T., & Lee, H. C. (2021). Coronary Large Conductance Ca(2+)-Activated K(+) Channel Dysfunction in Diabetes Mellitus. *Front Physiol*, *12*, 750618. https://doi.org/10.3389/fphys.2021.750618

Macvanin, M. T., Rizzo, M., Radovanovic, J., Sonmez, A., Paneni, F., & Isenovic, E. R. (2022). Role of Chemerin in Cardiovascular Diseases. *Biomedicines*, *10*(11), 2970. https://www.mdpi.com/2227-9059/10/11/2970

Méndez-Barbero, N., Gutiérrez-Muñoz, C., & Blanco-Colio, L. M. (2021). Cellular Crosstalk between Endothelial and Smooth Muscle Cells in Vascular Wall Remodeling. *International Journal of Molecular Sciences*, *22*(14), 7284. https://www.mdpi.com/1422-0067/22/14/7284

Neves, K. B., Lobato, N. S., Lopes, R. A., Filgueira, F. P., Zanotto, C. Z., Oliveira, A. M., & Tostes, R. C. (2014). Chemerin reduces vascular nitric oxide/cGMP signalling in rat aorta: a link to vascular dysfunction in obesity? *Clin Sci (Lond)*, *127*(2), 111-122. https://doi.org/10.1042/cs20130286

Nieves-Cintrón, M., Flores-Tamez, V. A., Le, T., Baudel, M. M.-A., & Navedo, M. F. (2021). Cellular and molecular effects of hyperglycemia on ion channels in vascular smooth muscle. *Cellular and Molecular Life Sciences*, *78*(1), 31-61. https://doi.org/10.1007/s00018-020-03582-z

Salvatore, T., Pafundi, P. C., Galiero, R., Rinaldi, L., Caturano, A., Vetrano, E., Aprea, C., Albanese, G., Di Martino, A., Ricozzi, C., Imbriani, S., & Sasso, F. C. (2021). Can Metformin Exert as an Active Drug on Endothelial Dysfunction in Diabetic Subjects? *Biomedicines*, *9*(1), 3. https://www.mdpi.com/2227-9059/9/1/3

Shen, W., Tian, C., Chen, H., Yang, Y., Zhu, D., Gao, P., & Liu, J. (2013). Oxidative stress mediates chemerin-induced autophagy in endothelial cells. *Free Radical Biology and Medicine*, *55*, 73-82. https://doi.org/https://doi.org/10.1016/j.freeradbiomed.2012.11.011

Stanke, F., Cracowski, J. L., Chavanon, O., Magne, J. L., Blin, D., Bessard, G., & Devillier, P. (1998). Glibenclamide inhibits thromboxane A2-induced contraction in human internal mammary artery and saphenous vein. *Eur J Pharmacol*, *341*(1), 65-71. https://doi.org/10.1016/s0014-2999(97)01458-1

Wang, M., Li, Y., Li, S., & Lv, J. (2022). Endothelial Dysfunction and Diabetic Cardiomyopathy. *Front Endocrinol (Lausanne)*, *13*, 851941. https://doi.org/10.3389/fendo.2022.851941

Zitron, E., Kiesecker, C., Lück, S., Kathöfer, S., Thomas, D., Kreye, V. A., Kiehn, J., Katus, H. A., Schoels, W., & Karle, C. A. (2004). Human cardiac inwardly rectifying current I Kir2. 2 is upregulated by activation of protein kinase A. *Cardiovascular Research*, *63*(3), 520-527.