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Research Article

Relaxation Responses of Ketamine and Propofol to Vasoactive Agents in Streptozotocin-Induced Diabetic Rats

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Summary: Diabetes mellitus (DM) is a major risk factor for the development of endothelial dysfunction which affects the ability of blood vessels to regulate vascular tone. The study aimed to investigate the mechanisms of vasodilator action of the anaesthetic agents ketamine and propofol in diabetic rat aorta. 30 male Sprague-Dawley rats were randomly divided into two equal groups: (i) non-diabetic control (ii) Streptozotocin-induced diabetic group. DM was induced by a single intra-peritoneal injection of streptozotocin at 50 mg/kg body weight. Blood samples were taken from the tail vein after 24 hours and tested for glucose level using an automated glucose analyser. A blood glucose ≥10 mmol/L confirmed hyperglycaemia and the development of DM. Rats were sacrificed, and the aortae excised. The vascular responses of aortic rings from both groups to ketamine, propofol in the presence of vasoactive agents were studied using standard organ bath procedures. Ketamine and propofol reduced Phe-induced contraction similarly in the diabetic and control groups. Barium chloride, attenuated the relaxation response to propofol in diabetic aorta when compared to ketamine. 4-aminopyridine significantly attenuated the relaxation response to ketamine and propofol in diabetic aorta. Glibenclamide, significantly reduced ketamine-induced relaxation in diabetic aorta when compared to propofol. Activation of K+ channels with nicorandil or NS1619 did not affect the relaxation response to ketamine or propofol in diabetic aorta. The results recommend that propofol can be effective in mitigating the consequences of hemodynamic instability in glibenclamide treated diabetics when compared to ketamine. This response is mediated by propofol-induced inhibition of intracellular calcium influx..

Keywords*: Ketamine, propofol, Vascular reactivity, Potassium Channel Modulators, Diabetes.*

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INTRODUCTION

Diabetes mellitus (DM) and the subsequent development of vascular dysfunction, is one of the leading causes of increased morbidity and mortality among affected humans and animals (Dhanavathy, 2015; Ding, & Tringle, 2005). The prolonged hyperglycaemia results in damage to organs including eyes, heart, kidneys and blood vessels, leading to increase vascular tone, which may also arise due to oxidative stress (Liwa *et al*., 2017; Reid *et al*., 2018). These are high risk factors that must be considered for the diabetic patients undergoing surgery due to hemodynamic instability and the high morbidity and mortality.

The anaesthetic induction agent propofol, produces vasodilation which appears to be caused by blockade of voltage-gated influx of extracellular calcium, independent of the endothelium (Chan *et al*., 2000; Kim *et al*., 2007; Schulingkamp *et al*., 2005), and endothelium-dependent mechanisms (Hao *et al*., 2017; Zhu *et al*., 2001). The vasodilatory effect of propofol is attributed to its ability to cause the production and release of NO from endothelial cells (Schulingkamp *et al*., 2005). On the other hand, ketamine, another anaesthetic agent, is known to cause vasoconstriction through depolarization of the voltage gated (Kv) potassium ion channels (Kim *et al*., 2007). Prior to this vasoconstriction phase, relaxation usually occurs but is short-lived. Its vascular relaxation effect is also independent of the endothelium and is thought to be due partly to alteration in calcium influx (Ibeawuchi *et al*., 2008).

Additionally, other studies have reported that ketamine directly inhibits KATP channel activities in non-diabetic tissues (Kawano *et al*., 2005, 2010). Interestingly, glibenclamide also inhibits KATP channel activities (Jackson, 2000), and is a treatment option for DM (Gribble and Reimann, 2003). Since diabetes affects potassium ion channels and ketamine inhibits KATP channels, it is important to understand if there is exaggeration in the vascular responses to ketamine when glibenclamide is the treatment option in DM.

MATERIALS AND METHODS

Experimental Animals; Male Sprague- Dawley rats (8-10 weeks old) weighing between 170g and 230g were obtained from the Animal House following ethical approval by the FMS/UWI Ethics Committee. Thirty rats were randomly assigned to two groups: (i) Streptozotocin (STZ)-treated, DM rats and (ii) Non-diabetic (control) rats. All animals were fed with standard rat chow and water *ad libitum*

Induction of Diabetes Mellitus; Sprague-Dawley rats assigned to the DM group were given a single intraperitoneal injection (i.p.) of 50 mg/kg body weight STZ (Sigma-Aldrich) dissolved in distilled water, according to previous method used (Dhanavathy, 2015). Basal glucose levels of all experimental animals were determined using an automated glucose analyser (Glucometer Acu-check mini plus, Roche, Germany) and blood glucose levels were assessed at 24-hour intervals following STZ injection. DM was confirmed when blood glucose level was equal to or greater than 10 mmol/L.

Preparation of Aortic Rings; STZ-induced diabetic and non-diabetic (control) rats were sacrificed by cervical dislocation and the thoracic aorta excised and placed in cold (4^0C) physiological Kreb's solution (PSS). Each aorta was cleaned of adhering fat and connective tissues and then cut into rings of 2-3 mm in length. Aortic rings were transferred to an organ bath containing PSS with the following composition (mM): NaCl, 112; KCl 5: $CaCl_2$, 1.8; $MgCl_2$, 1 $NaHCO_3$, 25; KH_2PO_4, 0.5; Glucose 10; and a mixture of 5% carbon dioxide and 95% oxygen at 37ºC passed into the solution to achieve and maintain a pH of 7.4. Aortic ring segments were mounted between two stainless steel wires at optimal length for isometric tension recording, utilizing an isometric force transducer (SS12LA, Biopac Systems Inc., Goleta, CA, USA) connected to a data acquisition unit (Biopac BSL PRO 7 computer software). The tissues were given a passive 1gram tension and allowed to equilibrate for at least 60 minutes. The aortic rings were then equilibrated for another 90 minutes while being rinsed with PSS every 10 minutes (Nwokocha *et al*., 2011, 2012). Following the equilibration period, 10^{-10}-10^{-4} phenylephrine (Phe from Sigma-Aldrich) was added cumulatively to the organ bath until a maximal contracted response was achieved. This plateau response was attained before any further additions of drugs. The ED_{70} concentration (10^{-6} M) obtained for Phe was determined and used throughout the remainder of the experiment.

When contracted rings had attained a steady state plateau, relaxation responses were recorded using cumulative concentrations of one of the following drugs: 10^{-10}-10^{-4} M Acetylcholine (ACh, Sigma-Aldrich), 10^{-9}-10^{-1} M ketamine (Sigma-Aldrich)), 10^{-9}-10^{-1} M propofol (Sigma-Aldrich), 10^{-4} M barium chloride (British Drug House Ltd), 10^{-3} M 4-AP (Sigma-Aldrich), 10^{-5} M glibenclamide (Sigma-Aldrich). Each concentration was added to the organ bath after the previous concentration showed no further change in tissue tension, that is, a steady state had been attained.

Data Analysis; Data were expressed as the mean tension ± SEM. The contractions induced by cumulative concentration of phenylephrine were expressed in terms of percentage of the maximum contraction obtained; while relaxation to the vasoactive agents were expressed as a percentage of the initial tension induced by Phe and the potassium channel modulators. Concentration response curves of each investigated drug were constructed after initial concentration reached a plateau. Statistical analysis of the data was performed using ANOVA, Repeated Measures of ANOVA and Post Hoc test. The Student's T-test was used where appropriate. A p-value <0.05 was considered significant.

RESULTS

Effect of Phenylephrine on Vascular Relaxation Responses to Ketamine and Propofol in Rat Aortic Rings.: Both ketamine (10^{-9} - 10^{-1} M) and propofol (10^{-9} - 10^{-1} M) significantly ($p< 0.5$) reduced contraction induced by Phe (10^{-6} M) at concentrations of 10^{-5} M and higher. However, there were no significant difference ($p> 0.5$) in the relaxation responses to either ketamine and propofol in diabetic group when compared to control group (Figures 1A and 1B). The values of pEC50 for ketamine (control vs. diabetic) were 3.90 ± 0.18 vs. 3.67 ± 0.09. Propofol, though not statistically significant, showed greater attenuation in relaxation in the diabetic group than ketamine in the same group. The pEC50 values were 5.05 ± 0.31 vs. 3.72 ± 0.04.

Effect of Barium Chloride on Vascular Relaxation Response to Ketamine and Propofol in Rat Aortic Rings: Relaxation response to ketamine following barium chloride-induced contraction at concentration (10^{-4} M), showed no significant difference in diabetic rat aorta when compared to the non-diabetic controls. The pEC50 values for control vs. diabetic were 3.55 ± 0.15 vs. 3.33 ± 0.20 as shown in figure 2A. However, the relaxation response to propofol showed significant attenuation in diabetic rat aortic rings compared to control. The pEC50 values control vs. diabetic were 4.77 ± 0.22 vs. 3.60 ± 0.14, p-value ≤ as shown in figure 2B. At concentration of 10-7 M, there was no significant difference with relaxation to propofol in diabetic when compared to control ($p>0.05$).

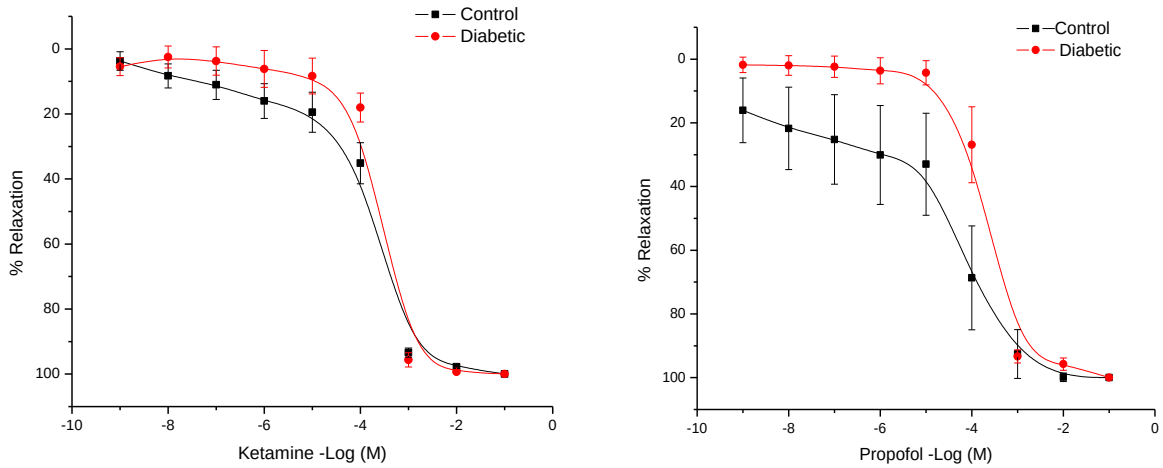

Figure 1
Concentration relaxation response curves for ketamine (1A) and propofol (1B) in diabetic (●) and non-diabetic control (■) rat aortic rings following Phe-induced contraction. Responses are expressed as a percentage of the maximum relaxation evoked by ketamine and propofol (10^{-1} M).

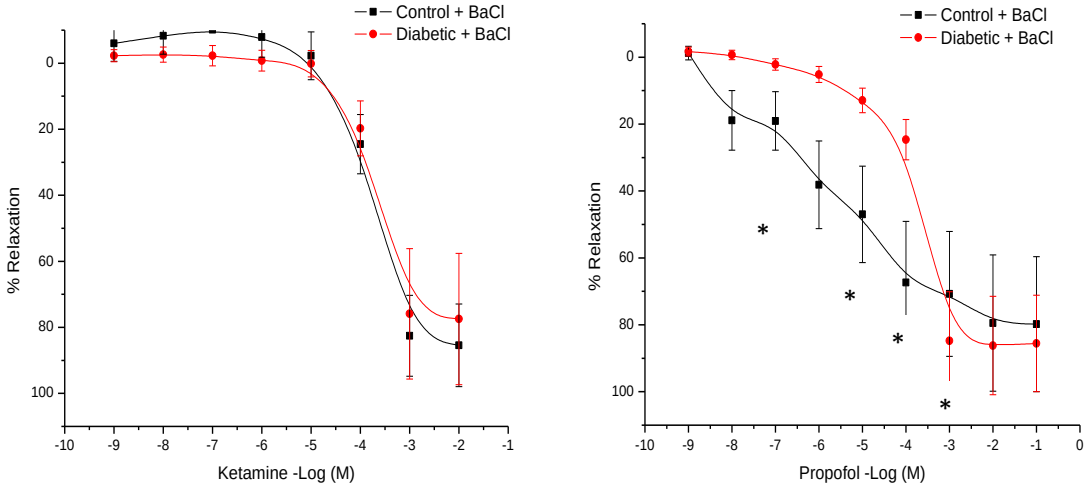

Figure 1
Concentration relaxation response curves for ketamine (A) and propofol (B) in the presence of barium chloride in diabetic (●) and control (■) Sprague-Dawley rat.

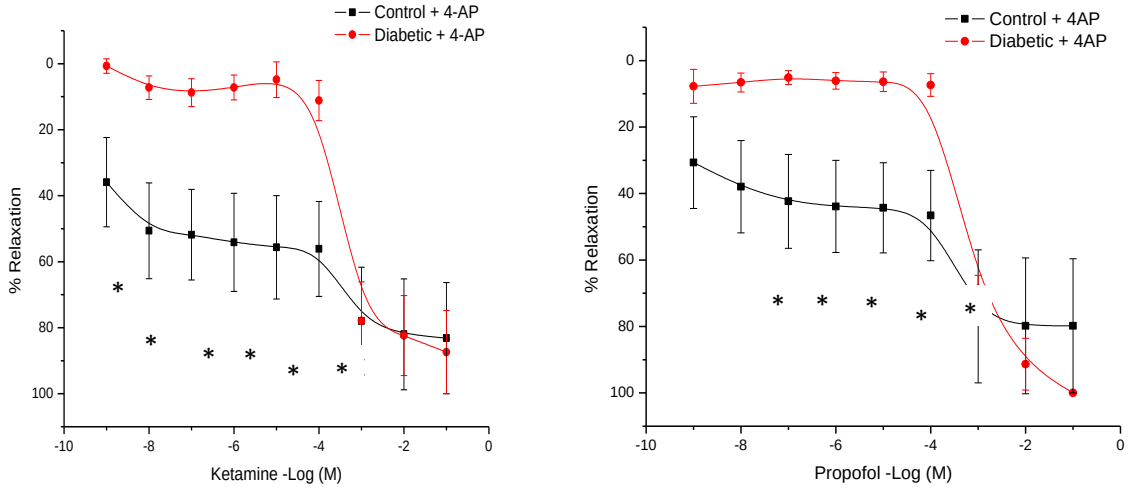

Figure 3
Concentration relaxation response curves for ketamine (3A) and propofol (3B) in the presence of4-AP in diabetic (●) and control (■) Sprague-Dawley rats

Effect of 4-AP on Vascular Relaxation Response to Ketamine and Propofol in Rat Aortic Rings: Relaxation response to ketamine and propofol in the presence of 4-AP (10^{-3} M), showed significant (p.<0,5) reduction in relaxation in the non-diabetic controls when compared to diabetic rat aortic rings . This resulted in a shift of the concentration-response curves to the right (Figures 3A and 3B). The pEC50 values for ketamine were 6.94 ± 0.45 vs.3.37 ± 0.15, p value ≤ 0.05. The values for propofol were 5.62 ± 0.35 vs. 2.78 ± 0.17.

Effect of Glibenclamide on Relaxation Response to Ketamine and Propofol Following Phe-induced Contraction in Rat Aortic Rings: Following pre-treatment of aortic rings with glibenclamide (10^{-4} M) for 15 minutes, then pre-contraction with Phe 10^{-6} M, cumulative concentrations of ketamine (10^{-9}-10^{-1} M) showed a significant (p<0.05) reduction in relaxation in diabetic rings treated with glibenclamide compared to diabetic rings without glibenclamide. The pEC50 values for diabetic rings without glibenclamide vs. diabetic with glibenclamide were 3.67 ± 0.09 vs. 3.11 ± 134.60. However, there was no significant difference between control treated with glibenclamide and diabetic rings treated with glibenclamide (Figure 4A). On the other hand, propofol showed no significant difference between any of the groups except for control without glibenclamide versus control + glibenclamide (Figure 4B).

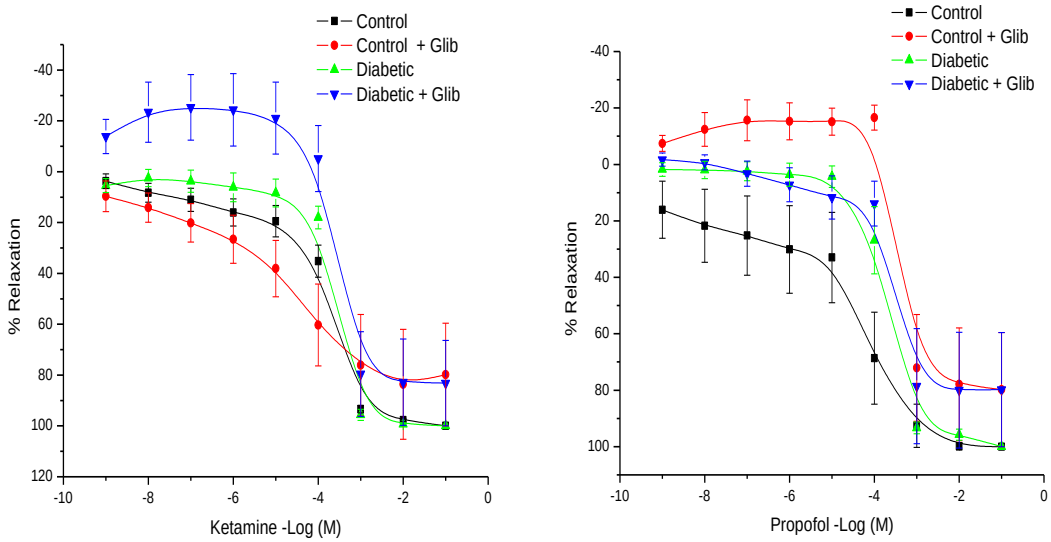

Figure 4:
Concentration relaxation curve of ketamine and propofol following blockade of K_{ATP} channels with glibenclamide in STZ-diabetic and non-diabetic (control) rat aortic rings.

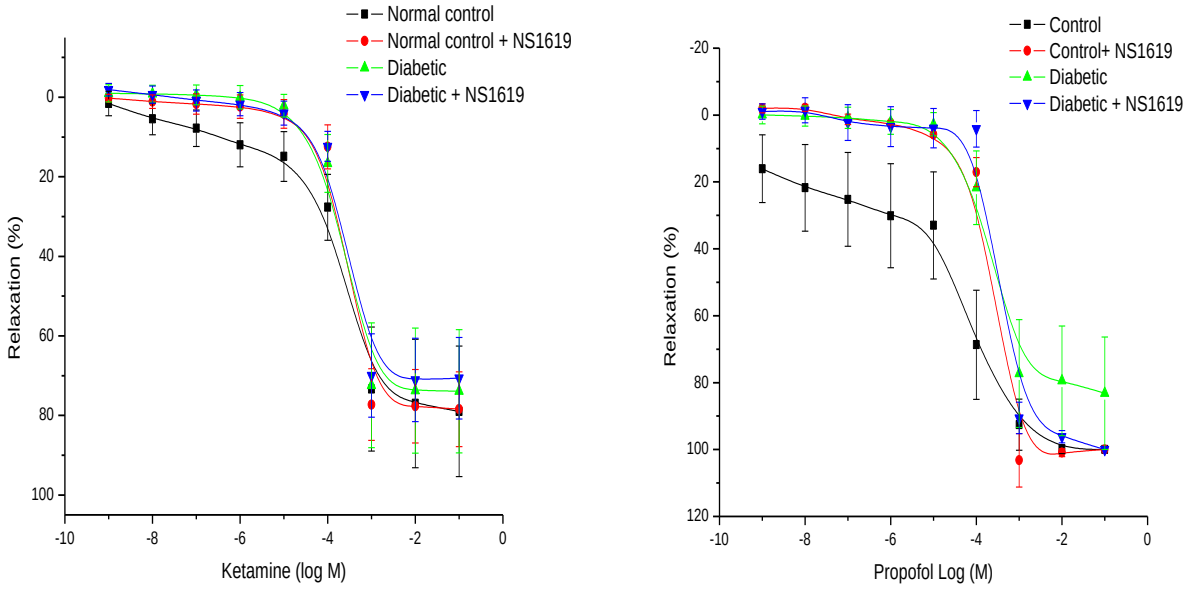

Figure 5:
Relaxation effects of ketamine and propofol in the presence of NS1619 applied to aortic rings obtained from diabetic and non-diabetic (control). Sprague-Dawley rats. N=6 (control) and N=5 (diabetic)

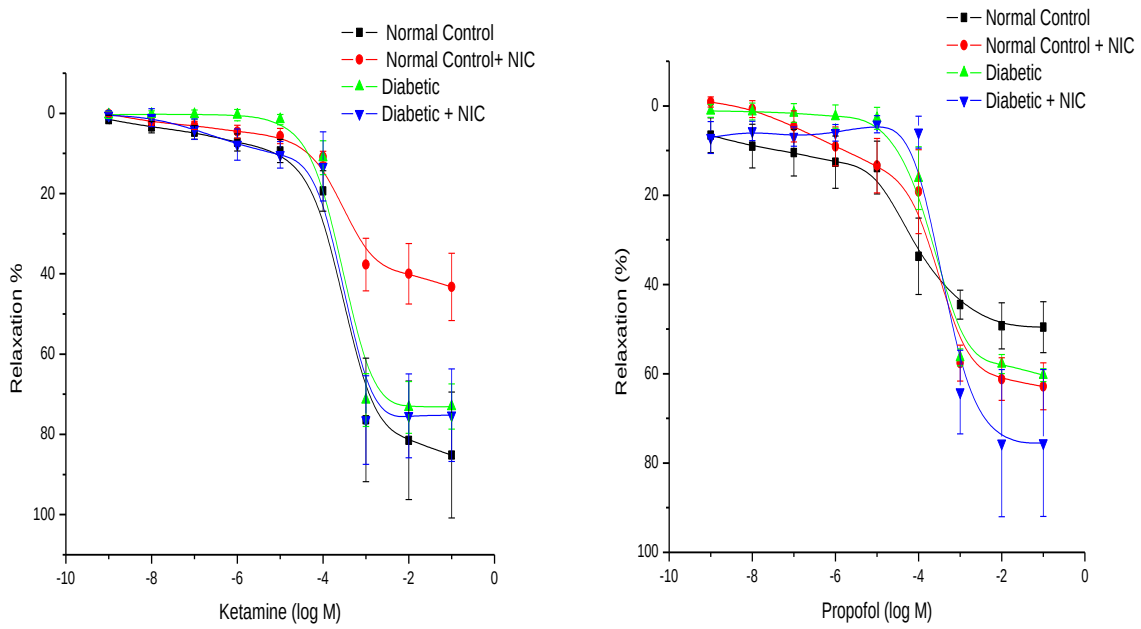

Figure 6:
Concentration response curves for ketamine and propofol in the presence of nicorandil applied to aortic rings obtained from STZ-diabetic (N=6) and non-diabetic (N=6) Sprague-Dawley rats.

Effect of NS1619 on Relaxation Response to Ketamine and Propofol in Rat Aortic Rings: Following pre-treatment with the BK_{Ca} potassium ion channel opener, NS1619 (10^{-7} M) prior to pre-contraction with Phe (10^{-6} M), there was no significant difference in the relaxation induced by ketamine or propofol between diabetic and non-diabetic rat aortic rings at the lower concentrations. However, both anaesthetic agents produced significant ($p< 0.05$) relaxation of the aortic rings at higher concentrations (10^{-4} to 10^{-1} M) Figure 5A and 5B.

Relaxation Response of Ketamine and Propofol in the Presence of Nicorandil: The relaxation effect of ketamine and propofol following activation of K_{ATP} channels with Nicorandil (10^{-6} M) prior to Phe pre-contraction, the relaxation response to ketamine and propofol was similar in both diabetic and non-diabetic rat aortic rings. As observed with NS1619, ketamine and propofol also showed significant ($p< 0.05$) relaxation at the higher concentration (10^{-4} to 10^{-1} M) Figure 6A and 6B respectively

DISCUSSION

The present study compared the vascular responses to ketamine and propofol in the presence of vasoactive agents in STZ-induced diabetic rat aorta. The main findings were that ketamine and propofol similarly reduced Phe-induced contraction in diabetic aorta when compared to control. The contractile response to Phe is increased in DM due to dysfunction in the alpha-adrenergic pathway (Chittari *et al*., 2010; Tsao *et al*., 2012), and an increase in the expression and affinity of adrenoceptors in STZ-induced diabetic rats (Potenza *et al*., 2009). This suggest that ketamine and propofol alter the calcium channels mediated calcium influx involved in the mechanism of Phe-induced contraction. The result further suggests that the reduced NO dependent vasorelaxation associated with diabetes is preserved by propofol, resulting in the similar response to the control group. This may be due to the NO releasing properties of propofol (Sobey, 2001), or that ketamine and propofol act at least in part, directly on VSM cells.

There is supporting evidence that DM progressively leads to impairment of potassium ion channels (Hao *et al*., 2017). These potassium ion channels are major contributors in the maintenance of vascular tone and are inclined towards constriction when there is impairment. Opening of potassium channels leads to efflux of potassium from VSM cells and closure of voltage-activated calcium channels. The closure of these calcium channels prevents calcium entry into cells leading to vasorelaxation, the converse leads to vasoconstriction (Hao *et al*., 2017). Hence, barium chloride blocks Kir channels, causing efflux of potassium from cells and a corresponding influx of calcium into cells, resulting in vasoconstriction. In the presence barium chloride-induced vasoconstriction, the vascular response to propofol in diabetic aorta was reduced, but remains unchanged with ketamine when compared to control. This demonstrates that diabetes impairs Kir channels which alters the vascular response to propofol due to increased calcium influx. Another finding showed significantly reduced relaxation responses to ketamine and propofol in the presence of 4-aminopyridine-induced vasoconstriction

in diabetic aorta. This demonstrates that Kv channels are also impaired in diabetes and supports an earlier study (Chai *et al*., 2005). This showed that the influx of calcium into cells reduced the relaxation response to ketamine and propofol. Propofol-induced relaxation has been attributed to a reduction in intracellular calcium within VSM cells (Sobey, 2001). A study reported a ketamine-induced inhibition of Kv channels resulting in an increase in vascular tone of rat mesenteric arteries (Kim *et al*., 2007). Information on the effects of ketamine on rat aorta is not readily available. However, the result of this study may suggest that ketamine has no significant inhibitory effect on Kv channels in rat aorta. This is evidenced by the similar relaxation responses when Kir channels are blocked.

Pre-incubation of KATP channels with glibenclamide showed a significant attenuation of ketamine relaxation response in diabetic rat aorta. Glibenclamide causes release of NO in cells with endothelium and blockade of calcium influx in cells without endothelium (Chan *et al*., 2000). This may suggest an endothelium dependent attenuated relaxation response to ketamine in the diabetic group due to impairment in glibenclamide-induced NO release. Glibenclamide acts as both a selective inhibitor of KATP channels (Jackson, 2000) in VSM cells and is a VSM relaxant (Ertuna and Yasa, 2005). When compared to ketamine, the vascular response to propofol was not altered in the diabetic rat aorta and indicates that KATP channels are not involved in propofol-induced relaxation. This result also suggests the possible release of propofol-induced NO, compensating for the lack of NO- induced endothelial dysfunction associated with diabetes (Pak *et al*., 2019). The potassium channel blockade with BaCl2 (a non-selective inward rectifier potassium channel blocker in rat aorta (Kir), or glibenclamide (KATP), elevated ketamine and propofol induced contractions in diabetic aortic rings, this is not in keeping with a relaxation or decreased in blood pressure, as may be expected for relaxation inducing agents (Romero *et al*., 2019; Cifuentes *et al*., 2018), and could suggest disruption to calcium channel responses in diabetes. This has previously been reported that both KATP and BKCa activities are attenuated in diabetes mellitus (Owu *et al*., 2013). This may have some clinical benefit as glibenclamide antagonize the protection of cardiac muscles when these channels are open (Tsao *et al*., 2012). However, this protective effect of KATP channel opening may be preserved in the diabetic patient being treated with glibenclamide and to whom propofol is also administered. In this case, propofol may be a better choice of anaesthetic. It has been reported that ketamine reduced the activity of KATP channels and inhibits relaxation induced by KATP channel openers (Kawano *et al*., 2010).

Additionally, the present study showed that opening of KATP and BKCa channels did not alter the vascular response to ketamine or propofol in diabetic aortic rings when compared to control. This suggest that opening of potassium channels preserves the relaxation response to ketamine and propofol in diabetes and stands as a treatment option in maintaining normal vascular tone. Therefore, the benefits derived from nicorandil in preventing angina will not be compromised in the diabetic patient to whom either ketamine or propofol is administered.

In conclusion, the results recommend that propofol can be effective in mitigating the consequences of hemodynamic instability in glibenclamide treated diabetics when compared to ketamine. This may be due to propofol-induced calcium modulation.

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