Title: **Reduction in the amplitude of shortening and Ca^{2+} transient by Phlorizin and Quercetin 3-O-Glucoside in ventricular myocytes from streptozotocin-induced diabetic rats**

Norin Nabil Hamouda [1], Muhammed Anwar Qureshi, Jumaa Musbah Alkaabi [2], Murat Oz[1], Frank Christopher Howarth[1]*

Departments of Physiology & [1]Pharmacology, [2]Internal Medicine, College of Medicine & Health Sciences, UAE University, Al Ain, UAE

***Address for correspondence:**

Professor Chris Howarth

Department of Physiology

College of Medicine & Health Sciences

UAE University

P.O. Box 17666

Al Ain

UAE

Tel: 00 9713 7137536

Email: chris.howarth@uaeu.ac.ae

Short title: **Effects of Phlorizin and Quercetin 3-O-Glucoside on diabetic ventricular myocytes.**

Summary

Diabetes mellitus is the leading cause of cardiovascular morbidity and mortality. Phlorizin (PHLOR) and Quercetin-3-O-Glucoside (QUER-3-G) are two natural compounds reported to have antidiabetic properties by inhibiting sodium/glucose transporters. Their effects on ventricular myocyte shortening and intracellular Ca^{2+} in streptozotocin (STZ) –induced diabetic rats were investigated. Video edge detection and fluorescence photometry were used to measure ventricular myocyte shortening and intracellular Ca^{2+}, respectively. Blood glucose in STZ rats was 4-fold higher (469.64±22.23 mg/dl, *n*=14) than in Controls (104.06±3.36 mg/dl, *n*=16). The amplitude of shortening was reduced by PHLOR in STZ (84.76±2.91%, *n*=20) and Control (83.72±2.65%, *n*=23) myocytes, and by QUER-3-G in STZ (79.12±2.28%, *n*=20) and Control (76.69±1.92%, *n*=30) myocytes. The amplitude of intracellular Ca^{2+} was also reduced by PHLOR in STZ (82.37±3.16%, *n*=16) and Control (73.94±5.22%, *n*=21) myocytes, and by QUER-3-G in STZ (73.62±5.83%, *n*=18) and Control (78.32±3.54%, *n*=41) myocytes. Myofilament sensitivity to Ca^{2+} was not significantly altered by PHLOR; however, it was reduced by QUER-3-G modestly in STZ myocytes and significantly in Controls. PHLOR and QUER-3-G did not significantly alter sarcoplasmic reticulum Ca^{2+} in STZ or Control myocytes. Altered mechanisms of Ca^{2+} transport partly underlie PHLOR and QUER-3-G negative inotropic effects in ventricular myocytes from STZ and Control rats.

Keywords: Diabetes mellitus, SGLT inhibitors, phlorizin, quercetin-3-O glucoside, ventricular myocytes, streptozotocin-induced diabetic rats

Introduction

Diabetes Mellitus (DM) is a major global health problem and thirty-five million people with DM live in the Middle East and North Africa. A variety of complications can arise from uncontrolled DM including cardiovascular diseases, neuropathy, nephropathy and retinopathy which makes it the leading cause of cardiovascular morbidity and mortality, blindness, end-stage renal failure and non-traumatic limb amputations (International Diabetes Federation 2013). Control of DM involves different approaches including lifestyle that involves increasing physical activity and weight loss, and therapeutic approaches that involve administration of medications (Ismail-Beigi 2012). The development of new antidiabetic medications without the side effects associated with the use of other available drugs such as weight gain and hypoglycemia as with insulin, sulphonylureas and thiazolidinediones, and diarrhea and nausea as with metformin and incretin mimetics, is a continuous challenge (Chao and Henry 2010). Sodium-glucose co-transporter (SGLT) inhibitors are a new class of antidiabetic drugs that work by a novel insulin-independent mechanism. SGLT inhibitors are subdivided into three subclasses including non-selective or dual SGLT1 and SGLT2 inhibitors such as Phlorizin (PHLOR) (Chao and Henry 2010) and LX4211 (Zambrowicz et al. 2012), selective SGLT1 inhibitors such as quercetin-3-Oglucoside (QUER-3-G) (Cermak et al. 2004; Gee et al. 1998), KGA-2727 (Shibazaki et al. 2012) and KGA-3235 (Kissei Pharmaceuticals 2009), and selective SGLT2 inhibitors such as dapagliflozin (Komoroski et al. 2009), canagliflozin (Sha et al. 2011), seragliflozin (Katsuno et al. 2007) and ipragliflozin (Imamura et al. 2012). PHLOR is a natural organic phenolic glycoside belonging to the chalcone class (Ehrenkranz et al. 2005;Tahrani et al. 2013). It was first isolated by French scientists in 1835 from apple tree bark. In 1886, von Mering observed that PHLOR produces glycosuria in doses above 1.0 g (Ehrenkranz et al. 2005). PHLOR is the parent compound from which synthesized SGLT inhibitors were derived (Tsujihara et al. 1996). PHLOR was not itself pharmaceutically developed as an SGLT inhibitor because it is poorly absorbed by the intestine. It is hydrolysed to another compound phloretin which is an inhibitor of facilitative glucose transporter 1 (GLUT1) and thus has low oral bioavailability (Chao and Henry 2010). QUER-3-G is a flavonol glycoside that is found in several plants including apple, tea, onion, crataegus, pistachio nut, saint John's wort in

addition to many fruits, vegetables and cereals (Razavi et al. 2009; Valentová et al. 2014; Wang et al. 2013). In addition to inhibiting SGLT1, QUER-3-G has been reported to also inhibit alpha-glucosidase enzyme (Li et al. 2009). Therefore, it has dual antidiabetic action which may make it a good parent for the development of antidiabetic drugs. Our previous study showed that the selective SGLT2 inhibitor dapagliflozin significantly reduced the amplitude of shortening and Ca^{2+} transient in myocytes from STZ rats (Hamouda et al. 2014). Therefore, we extended this study to investigate also the effects of other SGLT inhibitors, like PHLOR and QUER-3-G, on shortening and intracellular Ca^{2+} in ventricular myocytes from STZ-induced diabetic rats.

Materials and Methods

Ethical approval

This study was approved by the Animal Ethics Committee, College of Medicine & Health Sciences, United Arab Emirates University.

Experimental protocol

Experiments were performed in STZ-induced diabetic (*n*=14) and in age-matched Control rats (*n*=16). Diabetes was induced in 2 month old rats by injecting STZ (60 mg/kg, i.p.) dissolved in citrate buffer. Control rats received citrate buffer alone. Animals received standard rat chow and water *ad libitum*. Before each experiment, bodyweight, heart weight and non-fasting blood glucose were measured.

Ventricular myocyte isolation

Ventricular myocytes were isolated according to previously described techniques (Howarth et al. 2002). In brief, rats were euthanized by use of a guillotine. Hearts were then quickly removed and mounted on a Langendorff system for retrograde perfusion. Hearts were perfused at a constant flow rate of 8 ml.g $heart^{-1}.min^{-1}$ and at 35-36 ºC with cell isolation solution containing in mM/l: 130.0 NaCl, 5.4 KCl, 1.4 $MgCl_2$, 0.75 $CaCl_2$, 0.4 NaH_2PO_4, 5.0 HEPES, 10.0 glucose, 20.0 taurine and 10.0 creatine (pH 7.3) until heart contraction had stabilized. Perfusion was then switched for 4 min to Ca^{2+}-free cell

isolation solution containing 0.1 mM/l EGTA and then for 6 min to cell isolation solution containing 0.05 mM/l Ca^{2+}, 0.60 mg/ml type 1 collagenase (Worthington Biochemical Corp, Lakewood, NJ, USA) and 0.075 mg/ml type XIV protease (Sigma, Taufkirchen, Germany). Ventricle tissue was excised from the heart, minced carefully by the use of scissors and then gently agitated in collagenase-containing isolation solution supplemented with 1 % BSA. Cells were filtered from this solution at 4 min intervals and re-suspended in cell isolation solution containing 0.75 mM/l Ca^{2+}.

Measurement of ventricular myocyte shortening

Myocytes were allowed to settle on the glass bottom of a Perspex chamber mounted on the stage of an inverted microscope (Axiovert 35, Zeiss, Göttingen, Germany). Myocytes were superfused (3-5 ml/min) with normal Tyrode (NT) containing the following in mM/l: 140.0 NaCl, 5.0 KCl, 1.0 $MgCl_2$, 10.0 glucose, 5.0 HEPES, 1.8 $CaCl_2$ (pH 7.4). PHLOR (Sigma-Aldrich, Saint Louis, Missouri, USA) and QUER-3-G (Sigma, Taufkirchen, Germany) were dissolved in dimethyl sulphoxide (DMSO) to give a final concentration of 10^{-6} M. This concentration has been chosen based on a concentration-response experiment done in our previous study on dapaglilozin and to enable comparison with this study (Hamouda et al. 2014). The final concentration of DMSO in all experimental test solutions was 0.005%. It has been reported that 0.01% DMSO does not significantly affect contractile parameters in rat ventricular myocytes (Sun et al. 2010). Shortening of electrically stimulated (1 Hz) ventricular myocytes was recorded using a video edge detection system (VED-114, Crystal Biotech, Northborough, MA, USA) at 35-36 °C. Resting cell length (RCL), time to peak (TPK) shortening, time to half (THALF) relaxation and amplitude (AMP) of shortening (expressed as a percentage of shortening in Control and STZ myocytes perfused with NT) were measured. Data were acquired and analyzed with Signal Averager software v 6.37 (Cambridge Electronic Design, Cambridge, UK).

Measurement of intracellular Ca^{2+} concentration

Myocytes were loaded with the fluorescent indicator fura-2 AM (F-1221, Molecular Probes, Eugene, OR, USA) as previously described (Howarth et al. 2002). A

final fura-2 concentration of 2.5 µM/l was obtained by adding 6.25 µl of a 1.0 mM/l stock solution of fura-2 AM (dissolved in DMSO) to 2.5 ml of myocytes suspended in NT solution. Myocytes were loaded with fura-2 AM by gentle shaking for 10 min at room temperature (24 °C). Myocytes were then centrifuged, washed with NT to remove extracellular fura-2 and left for 30 min to ensure complete hydrolysis of the intracellular ester. Intracellular Ca^{2+} concentration was measured by illuminating myocytes alternately by 340 nm and 380 nm light using a monochromator (Cairn Research, Faversham, UK) which changed the excitation light every 2 ms. The resulting fluorescence emitted at 510 nm was recorded by a photomultiplier tube and the ratio of the emitted fluorescence at the two excitation wavelengths (340/380 ratio) was calculated to provide an index of intracellular Ca^{2+} concentration. Resting fura-2 ratio, TPK Ca^{2+} transient, THALF decay of the Ca^{2+} transient and the amplitude of the Ca^{2+} transient (expressed as a percentage of Ca^{2+} transient in Control and STZ myocytes perfused with NT) were measured in electrically stimulated (1 Hz) myocytes maintained at 35-36 °C. Data were acquired and analyzed with Signal Averager software v 6.37 (Cambridge Electronic Design, Cambridge, UK).

Assessment of myofilament sensitivity to Ca^{2+}

Shortening and fura-2 ratio were recorded simultaneously in some myocytes (Howarth et al. 2011). Myofilament sensitivity to Ca^{2+} was assessed from phase-plane diagrams of fura-2 ratio versus cell length by measuring the gradient of the fura-2 - cell length trajectory during late relaxation of the twitch contraction. The position of the trajectory reflects the relative myofilament response to Ca^{2+} and hence, can be used as a measure of myofilament sensitivity to Ca^{2+} (Howarth and Qureshi 2008; Spurgeon et al. 1992).

Measurement of sarcoplasmic reticulum Ca^{2+} release

Sarcoplasmic reticulum (SR) Ca^{2+} release was assessed according to previously described techniques (Bassani 1995; Howarth et al. 2002; Howarth et al. 1999). After establishing steady state Ca^{2+} transients in electrically stimulated (1 Hz) myocytes maintained at 35-36 °C and loaded with fura-2, stimulation was paused for a period of 5

sec. Caffeine (20 mM) was then applied rapidly using a solution switching device (Levi et al. 1996) for 10 sec. Electrical stimulation was then resumed. SR releasable Ca^{2+} was assessed by measuring the amplitude of the caffeine-stimulated Ca^{2+} transient. Fractional release of SR Ca^{2+} was assessed by comparing the amplitude of the electrically-stimulated steady state Ca^{2+} transients with that of the caffeine-stimulated Ca^{2+} transient. Refilling of SR was assessed by measuring the rate of recovery of electrically-stimulated Ca^{2+} transients following application of caffeine.

Statistical Analysis

Results are expressed as the mean ± SEM of 'n' observations. 'n' refers to number of rats or ventricular myocytes. Data were analyzed by either the Independent samples t-test or one-way ANOVA with Bonferroni *post hoc* for multiple comparisons as appropriate using IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp., NY, USA). Statistical significance was set at $P < 0.05$ and was denoted by horizontal lines on all graphs.

Results

General characteristics of streptozotocin-induced diabetic rats

Experiments began 2 months after STZ treatment. At the time of experiments, non-fasting glucose level was measured in blood taken from the tail vein to confirm diabetes. STZ rats had elevated non-fasting blood glucose level (469.64 ± 22.23 mg/dl, n=14) compared to Controls (104.06 ± 3.36 mg/dl, n=16). STZ rats had significantly (P<0.05) lower body weights (267.57 ± 7.45 g, n=14) compared to Controls (352.25 ± 9.09 g, n=16), as well as significantly (P<0.05) lower heart weights (1.06 ± 0.02 g, n=14) compared to Controls (1.2 ± 0.03 g, n=16). Heart weight to body weight ratio of STZ rats was significantly (P<0.05) higher (4.00 ± 0.11 mg/g, n=14) compared to Controls (3.42 ± 0.05 mg/g, n=16).

Effects of Phlorizin and Quercetin-3-O-Glucoside on ventricular myocyte shortening

Ventricular myocyte shortening was assessed in electrically stimulated (1 Hz) STZ and Control myocytes maintained at 35-36°C. Figure 1 shows typical records of shortening in a Control myocyte during superfusion with NT, 5 min NT + PHLOR (Figure 1A) or NT + QUER-3-G (Figure 1B) and after 5 min washout with NT. The effects of PHLOR and QUER-3-G on RCL, TPK shortening, THALF relaxation and amplitude of shortening are shown in Figures 1C-J. RCL was not significantly ($P>0.05$) altered in STZ myocytes compared to Controls or by PHLOR and QUER-3-G (Figure 1C & G). TPK shortening was generally prolonged in STZ myocytes compared to Controls (Figure 1D & H). PHLOR had no significant ($P>0.05$) effect on TPK shortening (Figure 1D); however, TPK was significantly ($P<0.05$) shortened in STZ/QUER-3-G and in Control/QUER-3-G compared to STZ and Control myocytes (Figure 1H). THALF relaxation of shortening was not altered in STZ compared to Control or by PHLOR and QUER-3-G (Figure 1E & I). Amplitude of shortening was not significantly ($P>0.05$) altered in STZ myocytes compared to Controls (Figure 1F & J). Amplitude of shortening was significantly ($P<0.05$) reduced in STZ/PHLOR (84.76±2.91%, $n=20$) and Control/PHLOR (83.72±2.65%, $n=23$) myocytes compared to STZ and Control myocytes (Figure 1F). Amplitude of shortening was also significantly ($P<0.05$) reduced in STZ/QUER-3-G (79.12±2.28%, $n=20$) and Control/QUER-3-G (76.69±1.92%, $n=30$) myocytes compared to STZ and Control myocytes (Figure 1J).

Effects of Phlorizin and Quercetin-3-O-Glucoside on ventricular myocytes intracellular Ca^{2+}

Intracellular Ca^{2+} was measured in fura-2 loaded, electrically stimulated (1 Hz) myocytes at 35-36 °C. Figure 2 shows typical records of Ca^{2+} transient in a Control myocyte during superfusion with NT and NT + PHLOR (Figure 2A) or NT + QUER-3-G (Figure 2B) for 5 min. The effects of PHLOR and QUER-3-G on resting fura-2 ratio, TPK Ca^{2+} transient, THALF decay of the Ca^{2+} transient and amplitude of the Ca^{2+} transient are shown in Figures 2C-J. Resting fura-2 ratio was not significantly ($P>0.05$)

altered in STZ myocytes compared to Controls or by PHLOR (Figure 1C). QUER-3-G showed a significant (P>0.05) increase in Control/QUER-3-G myocytes (0.757±0.025, n = 41) compared to Controls (0.661±0.018, n = 41), but there was no significant (P>0.05) effect in STZ/QUER-3-G compared to STZ myocytes (Figure 1G). TPK Ca^{2+} transient was not significantly (P>0.05) altered in STZ myocytes compared to Controls and was not altered by either PHLOR or QUER-3-G (Figures 2D & H). THALF decay of the Ca^{2+} transient was also not significantly (P>0.05) altered in STZ compared to Control myocytes (Figures 2E & I). THALF decay of the Ca^{2+} transient was not significantly altered by PHLOR (Figure 2E); however, it was significantly (*P*<0.05) shortened by QUER-3-G in STZ/QUER-3-G and Control/QUER-3-G myocytes compared to STZ myocytes and Controls (Figure 2I) Amplitude of the Ca^{2+} transient was not significantly (P>0.05) altered in STZ myocytes compared to Controls (Figures 2F & J). However, the amplitude of the Ca^{2+} transient was significantly (*P*<0.05) reduced in STZ/PHLOR (82.37±3.16%, *n*=16) and Control/PHLOR (73.94±5.22%, *n*=21) myocytes compared to STZ and Control myocytes (Figure 2F). Amplitude of shortening was also significantly (*P*<0.05) reduced in STZ/QUER-3-G (73.62±5.83%, *n*=18) and Control/QUER-3-G (78.32±3.54%, *n*=41) myocytes compared to STZ and Control myocytes (Figure 2J).

Effects of Phlorizin and Quercetin-3-O-Glucoside on ventricular myocyte myofilament sensitivity to Ca^{2+}

In some myocytes shortening and Ca^{2+} transient were recorded simultaneously to measure myofilament sensitivity to Ca^{2+}. A simultaneous recording of shortening and Ca^{2+} transient in a Control myocyte is shown in Figure 3A and a phase plane diagram showing the relationship between the fura-2 ratio and shortening is shown in Figure 3B. The gradient of the fura-2-cell length trajectory was used to assess the relative myofilament response and sensitivity to Ca^{2+}. There was no significant difference in myofilament sensitivity to Ca^{2+} in myocytes from STZ compared to Control myocytes (Figures 3C & D) or in STZ/PHLOR and Control/PHLOR compared to STZ and Control myocytes (Figure 3C). There was a modest reduction in myofilament sensitivity to Ca^{2+}

in STZ/QUER-3-G and a significant (P<0.05) reduction in myofilament sensitivity in Control/QUER-3-G compared to STZ and Control myocytes (Figure 3D).

Effects of Phlorizin and Quercetin-3-O-Glucoside on ventricular myocytes sarcoplasmic reticulum Ca^{2+} release

SR Ca^{2+} release was investigated in fura-2 loaded STZ and Control myocytes maintained at 35-36 °C. Figure 4A shows a typical record of Ca^{2+} transients in a Control myocyte. The protocol included a period of electrical stimulation, a brief pause followed by rapid application of caffeine (20 mM) for 10 sec and then resumption of electrical stimulation. The protocol was repeated in each myocyte during superfusion with NT and then again following a 5 min superfusion with either NT + PHLOR or QUER-3-G. Amplitudes of the caffeine-evoked Ca^{2+} transient (Figures 4B & E), fractional release of Ca^{2+} (amplitude of the electrically stimulated Ca^{2+} transient / caffeine stimulated Ca^{2+} transient) (Figures 4 C & F) and recovery of the Ca^{2+} transient after caffeine and after resumption of electrical stimulation (Figures 4D & G) were not significantly (P>0.05) altered in STZ compared to Control or by PHLOR or QUER-3-G.

Discussion

Similar to findings in previous studies, STZ-induced DM was characterized by a 4-fold increase in blood glucose, lower body and heart weights and higher heart weight to bodyweight ratio compared to Control rats (Bracken et al. 2006; Howarth et al. 2002; Howarth and Qureshi 2001; Howarth et al. 2007). In the heart, the time course of myocyte shortening was prolonged in STZ compared to Control rats (Kotsanas et al. 2000; Singh et al. 2006). This study investigated the effects of PHLOR and QUER-3-G on contractility and Ca^{2+} transport in ventricular myocytes from STZ-induced diabetic rats. The main findings of the study were: (i) PHLOR and QUER-3-G decreased the amplitude of shortening in STZ and Control myocytes, (ii) PHLOR and QUER-3-G decreased the amplitude of the Ca^{2+} transient in STZ and Control myocytes, (iii) PHLOR did not alter myofilament sensitivity to Ca^{2+} in STZ and Control myocytes; however,

QUER-3-G decreased myofilament sensitivity to Ca^{2+} significantly in Control myocytes and modestly in STZ myocytes, (iv) PHLOR and QUER-3-G did not significantly alter fractional release of SR Ca^{2+} in STZ or Control myocytes.

PHLOR has been shown to have several beneficial cardioprotective effects. *In vivo* treatment of PHLOR prevented diabetic cardiomyopathy in *db/db* mice by changing the expression of some cardiac damaging proteins, and adjusting cardiac energy metabolism and lipids (Cai et al. 2013). PHLOR has also been shown to exert partial cardioprotection against isoproterenol-induced myocardial necrosis (Gupta et al. 2013) and to prevent electrically-induced ventricular tachyarrhythmia in guinea pig hearts during ischemia (Hirose et al. 2014). Previous studies have reported the effects of PHLOR on healthy ventricular myocyte shortening and intracellular Ca^{2+} (Olson et al. 2007); however, the effects of PHLOR on myocytes from diabetic heart remain to be clarified. QUER-3-G has been shown to have anticancer (Sudan & Rupasinghe, 2014), antidiabetic, antioxidant (Panda & Kar, 2007) and anti-allergic (Makino et al., 2013) effects; however, its effects on the heart also remain to be clarified. PHLOR and QUER-3-G decreased the amplitude of shortening to similar extents in ventricular myocytes in STZ and Control rats. Since cardiac contraction is regulated by intracellular Ca^{2+} levels the reductions in amplitude of shortening might be partly attributed to the action of PHLOR and QUER-3-G on intracellular Ca^{2+}. PHLOR and QUER-3-G decreased the amplitude of the Ca^{2+} transient to similar extents in myocytes from STZ and Control rats suggesting that alterations in Ca^{2+} transport might partly underlie the reductions in myocyte shortening.

STZ-induced diabetic rats develop cardiovascular complications in a short period of time starting with reduced heart rate 5-7 days after injection, reduced mean arterial pressure 14 days after injection, and decreased heart rate and mean arterial pressure variability 30-45 days after injection (Schaan et al. 2004). Although, experiments were carried out after 2 months of STZ injection allowing all the mentioned complications to develop, the extent of the observed effects of PHLOR and QUER-3-G on myocyte shortening and intracellular Ca^{2+} were similar suggesting that PHLOR and QUER-3-G affect healthy and diabetic cells in the same manner.

Further experiments were performed to investigate whether the change in the amplitude of shortening caused by PHLOR and QUER-3-G could be partly attributed to altered myofilament sensitivity to Ca^{2+}. Myofilament sensitivity to Ca^{2+} was not significantly altered by PHLOR in STZ and Control myocytes. However, QUER-3-G decreased myofilament sensitivity to Ca^{2+} in ventricular myocytes from Control rats and to a smaller extent in myocytes from STZ rats. This suggests that the effects of QUER-3-G might be partly attributed to changed myofilament sensitivity to Ca^{2+}.

PHLOR and QUER-3-G had no effects on SR Ca^{2+}, fractional release of Ca^{2+} or recovery of the Ca^{2+} transient after application of caffeine in either STZ or Control myocytes suggesting that the effects of PHLOR and QUER-3-G on myocyte shortening are unlikely to be related to an effect of these compounds on SR Ca^{2+} transport. In a previous study, Olson and colleagues demonstrated that PHLOR modestly decreased the amplitude and area of the Ca^{2+} transients. They also found that PHLOR did not significantly change SR Ca^{2+} load after caffeine application which is consistent with the current study (Olson et al. 2007).

Recent studies have shown that the selective SGLT2 inhibitor Dapagliflozin, an analogue of PHLOR (Meng et al. 2008), reduced the amplitude of shortening in a dose dependent manner, reduced the amplitude of the Ca^{2+} transient and reduced the amplitude of L-type Ca^{2+} current over a range of test potentials in rat STZ and Control myocytes (Hamouda et al. 2015). Further experiments would be required to establish whether alterations in L-type Ca^{2+} current are also involved in the negative inotropic effects of PHLOR and QUER-3-G.

Overall, PHLOR and QUER-3-G both reduced the amplitude of shortening to similar extents in STZ and Control myocytes. Owing to their negative inotropic effects, it is suggested that PHLOR and QUER-3-G might have a wide range of clinical applications in cardiovascular diseases such as angina, arrhythmias and hypertension. Considering their antidiabetic effects, they would be of a great benefit to diabetic patients who are prone to develop angina, myocardial infarction and congestive heart failure (International Diabetes Federation 2013).

Declaration of Competing Interests: The authors declare no conflicts of interest. The pharmaceutical industry has no influence or input in this scientific work.

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Figure Legends

Figure 1 – Effects of Phlorizin and Quercetin-3-O-Glucoside on shortening in ventricular myocytes from Streptozotocin-induced diabetic and Control rats.

Typical records of shortening in a Control myocyte superfused with normal Tyrode (NT), NT + 10^{-6} M PHLOR and NT during washout **(A)** and in another Control myocyte superfused with NT, NT + 10^{-6} M QUER-3-G and NT during washout **(B)**. PHLOR Graphs showing the mean resting cell length **(C**), time to peak (TPK) shortening **(D)**, time to half (THALF) relaxation of shortening **(E)** and amplitude (AMP) of shortening **(F)**. QUER-3-G Graphs showing the mean resting cell length **(G**), time to peak (TPK) shortening **(H)**, time to half (THALF) relaxation of shortening **(I)** and amplitude (AMP) of shortening **(J)**. Data are mean + S.E.M., *n*=20-23 myocytes from 7 rats for PHLOR and *n*=20-30 myocytes from 5-9 rats for QUER-3-G. Horizontal lines above the bars represent significant differences at the level of $P < 0.05$.

Figure 2 – Effects of Phlorizin and Quercetin-3-O-Glucoside on intracellular Ca^{2+} in ventricular myocytes from Streptozotocin-induced diabetic rats.

Typical records of Ca^{2+} transient in a Control myocyte superfused with normal Tyrode (NT) then NT + 10^{-6} M PHLOR **(A)** and in another Control myocyte superfused with NT then NT+ 10^{-6} M QUER-3-G **(B)**. PHLOR graphs showing the mean resting fura-2 ratio **(C)**, time to peak (TPK) Ca^{2+} transient **(D)**, time to half (THALF) decay of the Ca^{2+} transient **(E)** and amplitude (AMP) of the Ca^{2+} transient **(F)**. QUER-3-G graphs showing the mean resting fura-2 ratio **(G)**, time to peak (TPK) Ca^{2+} transient **(H)**, time to half (THALF) decay of the Ca^{2+} transient **(I)** and amplitude (AMP) of the Ca^{2+} transient **(J)**.

Data are mean + S.E.M., *n*=16-23 myocytes from 3-4 rats for PHLOR and *n*=18-41 myocytes from 3-6 rats for QUER-3-G. Horizontal lines above the bars represent significant differences at the level of $P < 0.05$.

Figure 3 – Effects of Phlorizin and Quercetin-3-O-Glucoside on myofilament sensitivity to Ca^{2+} in ventricular myocytes from Streptozotocin-induced diabetic and Control rats.

Typical records of shortening and Ca^{2+} transient recorded simultaneously in an electrically stimulated ventricular myocyte from a Control rat **(A)**. Typical phase plane diagram of fura-2 ratio unit (RU) vs. cell length in a myocyte from a Control rat. The arrow indicates the region where the gradient was measured **(B)**. Graph showing mean gradient of the fura-2 cell length trajectory during late relaxation of the twitch contraction during the period 500-800 ms with PHLOR **(C)** and QUER-3-G **(D)**. Data are mean + S.E.M., *n*=10-12 myocytes from 3-4 rats for PHLOR and *n*=12-29 myocytes from 3-6 rats for QUER-3-G.

Figure 4 – Effects of Phlorizin and Quercetin-3-O-Glucoside on sarcoplasmic reticulum Ca^{2+} release in ventricular myocytes from Streptozotocin-induced diabetic and Control rats.

Typical recording showing protocol employed in a Control ventricular myocyte during SR Ca^{2+} experiments **(A)**. Initially Ca^{2+} transients were recorded during electrical stimulation. Electrical stimulation was then paused for 5 sec and 20 mM caffeine was rapidly applied for 10 sec. After application of caffeine electrical stimulation was resumed. PHLOR graphs showing mean amplitude of caffeine -stimulated Ca^{2+} transients **(B)**, fractional release of Ca^{2+} **(C)**, and recovery of Ca^{2+} transients following rapid

application of caffeine **(D)**. QUER-3-G graphs showing mean amplitude of caffeine - stimulated Ca^{2+} transients **(E)**, fractional release of Ca^{2+} **(F)**, and recovery of Ca^{2+} transients following rapid application of caffeine **(G)**. Data are mean + S.E.M., *n*=13-16 myocytes from 3-4 rats for PHLOR and *n*=13-31 myocytes from 3-6 rats for QUER-3-G.

Figures

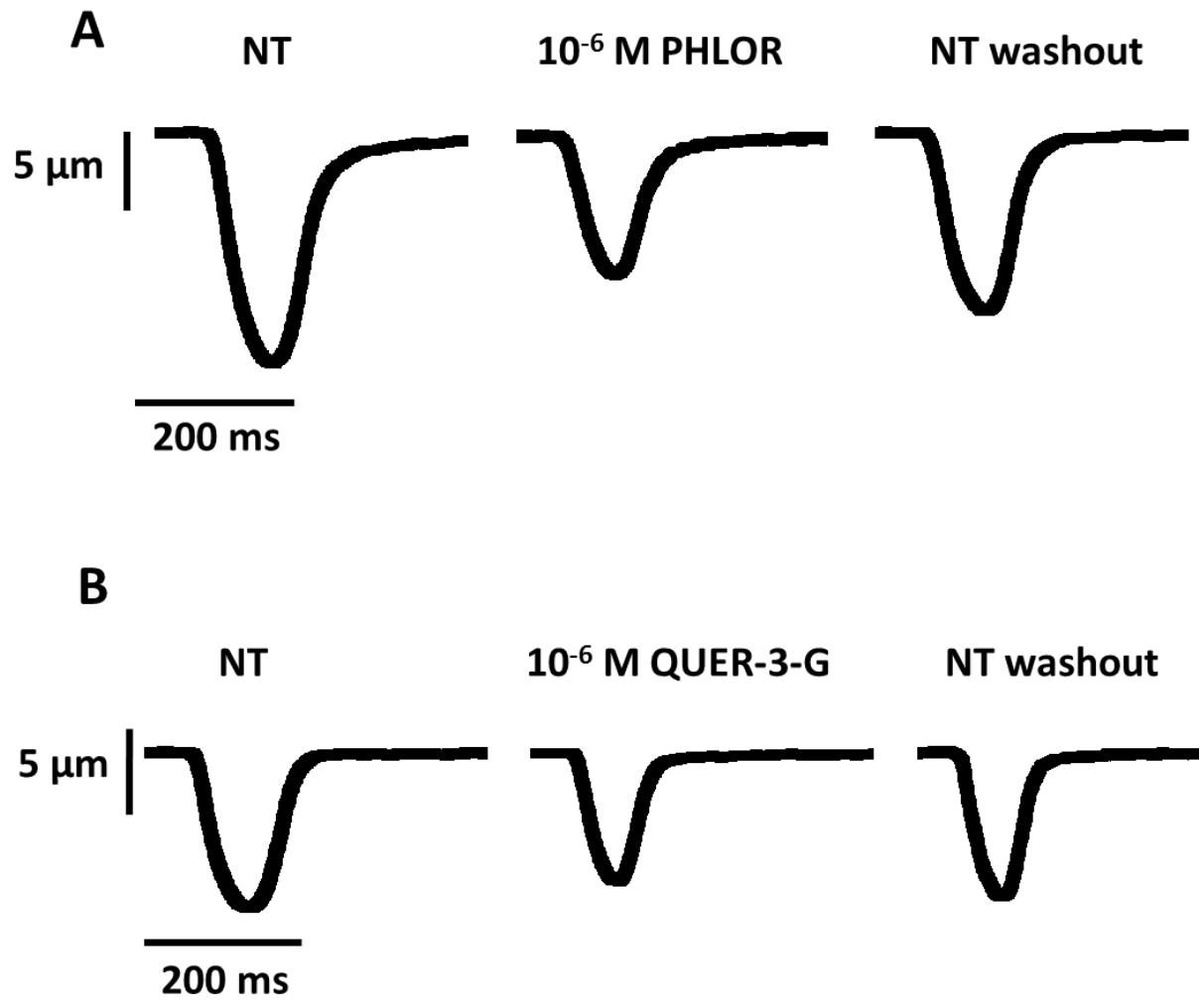

Figure 1A & B

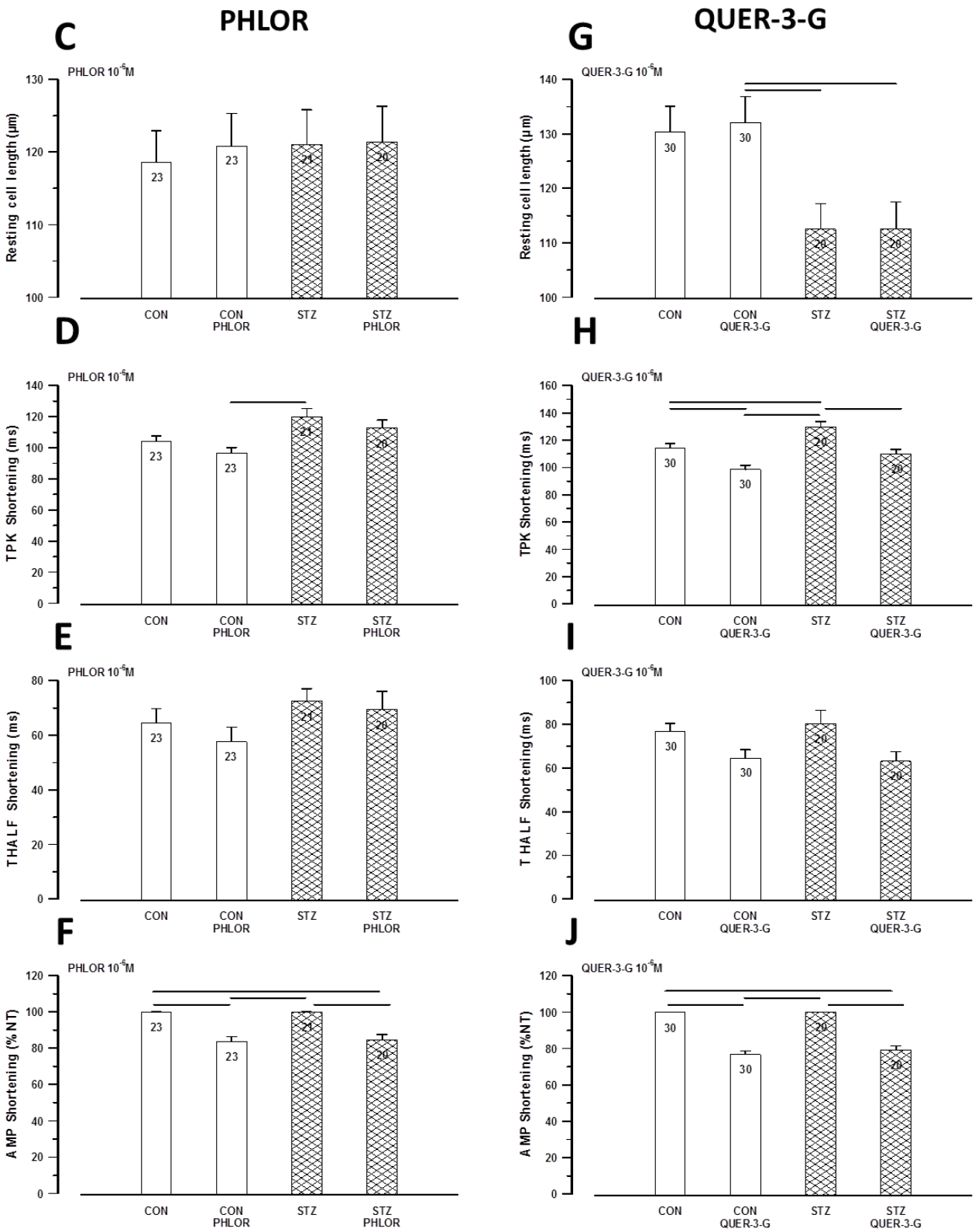
PHLOR
QUER-3-G
C
G
PHLOR 10-5M
QUER-3-G 10-5M
Resting cell length (µm)
D
H
TPK Shortening (ms)
E
I
THALF Shortening (ms)
F
J
AMP Shortening (%NT)
CON
CON PHLOR
STZ
STZ PHLOR
CON QUER-3-G
STZ QUER-3-G

Figure 1C-J

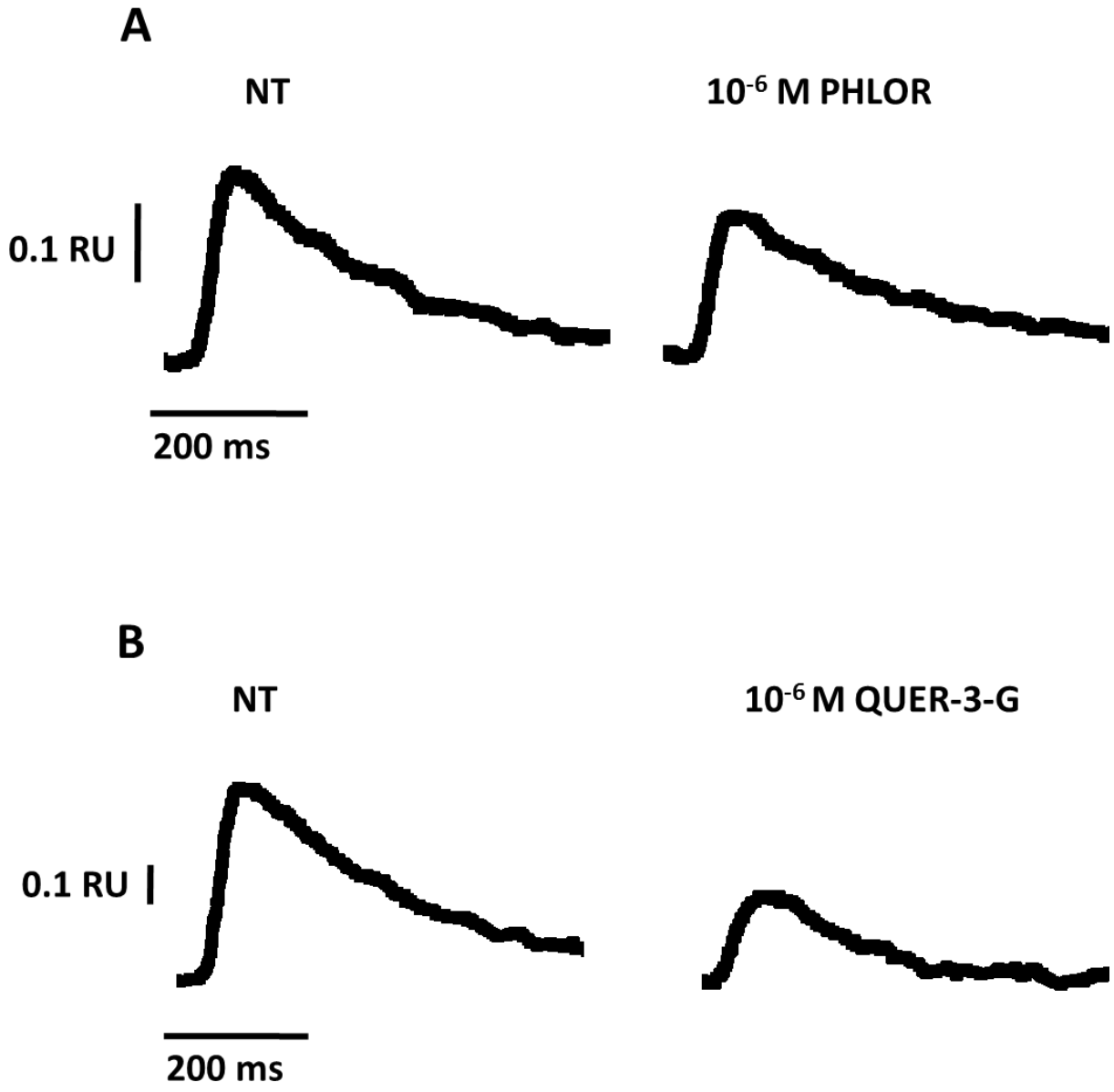

Figure 2A & B

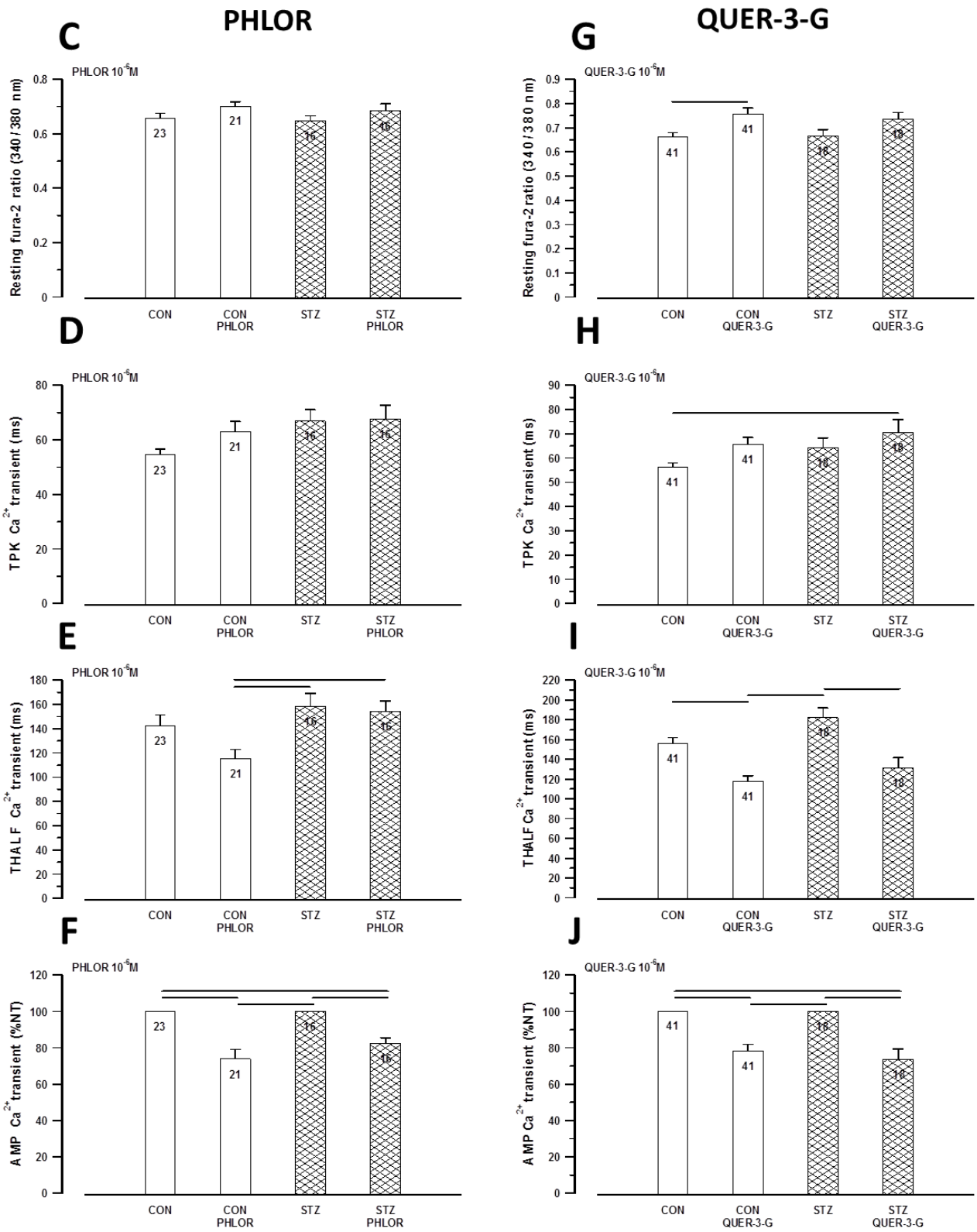
PHLOR
QUER-3-G
C
G
PHLOR 10-5M
QUER-3-G 10-5M
Resting fura-2 ratio (340/380 nm)
D
H
TPK Ca2+ transient (ms)
E
I
THALF Ca2+ transient (ms)
F
J
AMP Ca2+ transient (%NT)
CON
CON PHLOR
STZ
STZ PHLOR
CON QUER-3-G
STZ QUER-3-G

Figure 2C-J

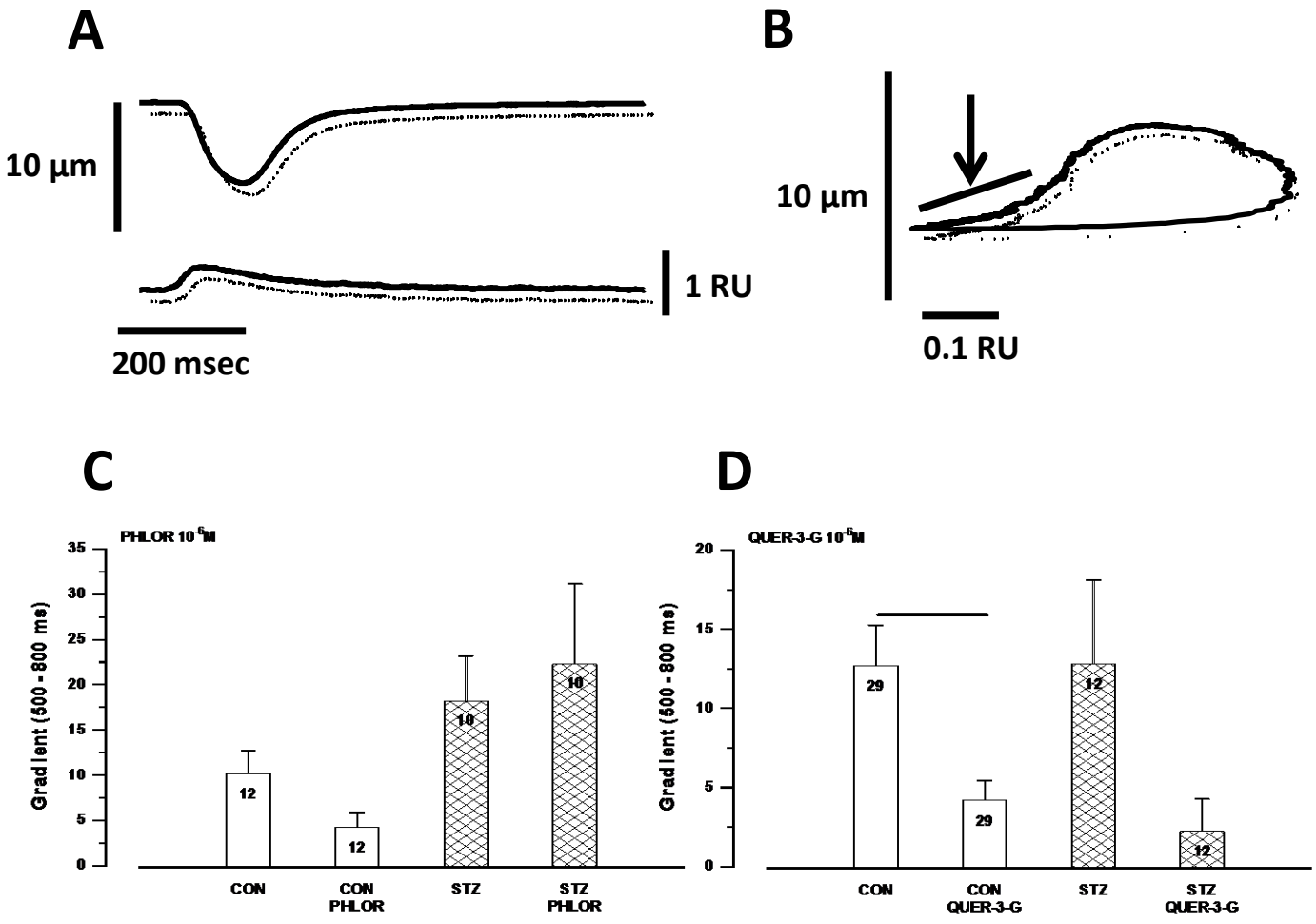
A
10 µm
1 RU
200 msec
B
10 µm
0.1 RU
C
PHLOR 10⁻⁶M
Gradient (500 - 800 ms)
35
30
25
20
15
10
5
0
12
12
10
10
CON
CON PHLOR
STZ
STZ PHLOR
D
QUER-3-G 10⁻⁶M
Gradient (500 - 800 ms)
20
15
10
5
0
29
29
12
12
CON
CON QUER-3-G
STZ
STZ QUER-3-G

Figure 3A-D

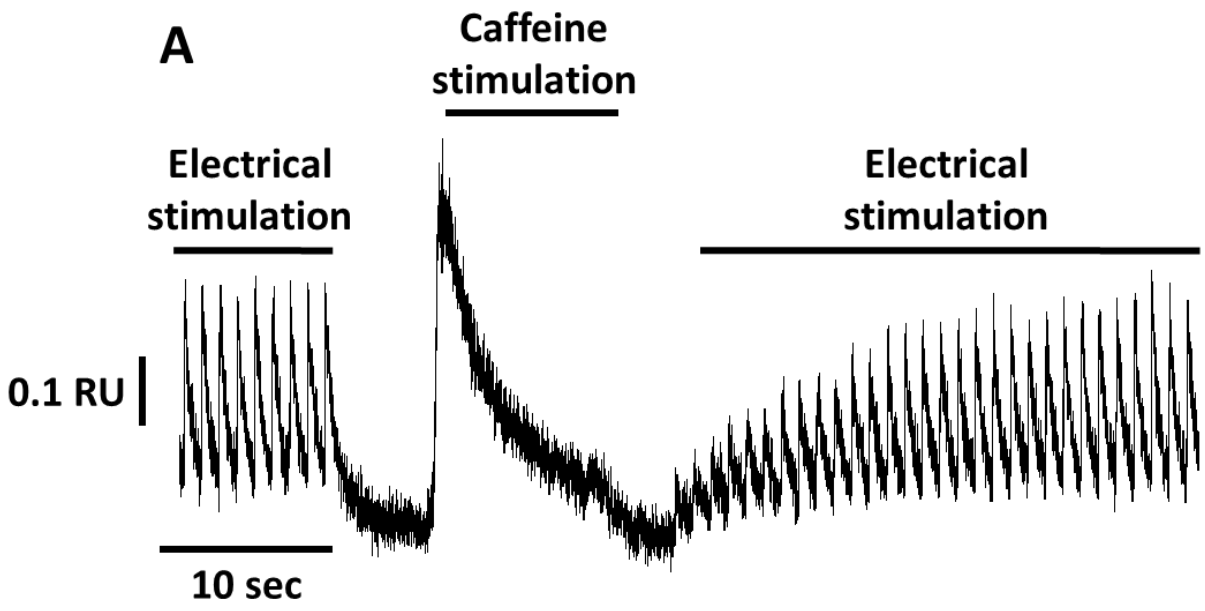
A
Caffeine stimulation
Electrical stimulation
Electrical stimulation
0.1 RU
10 sec

Figure 4A

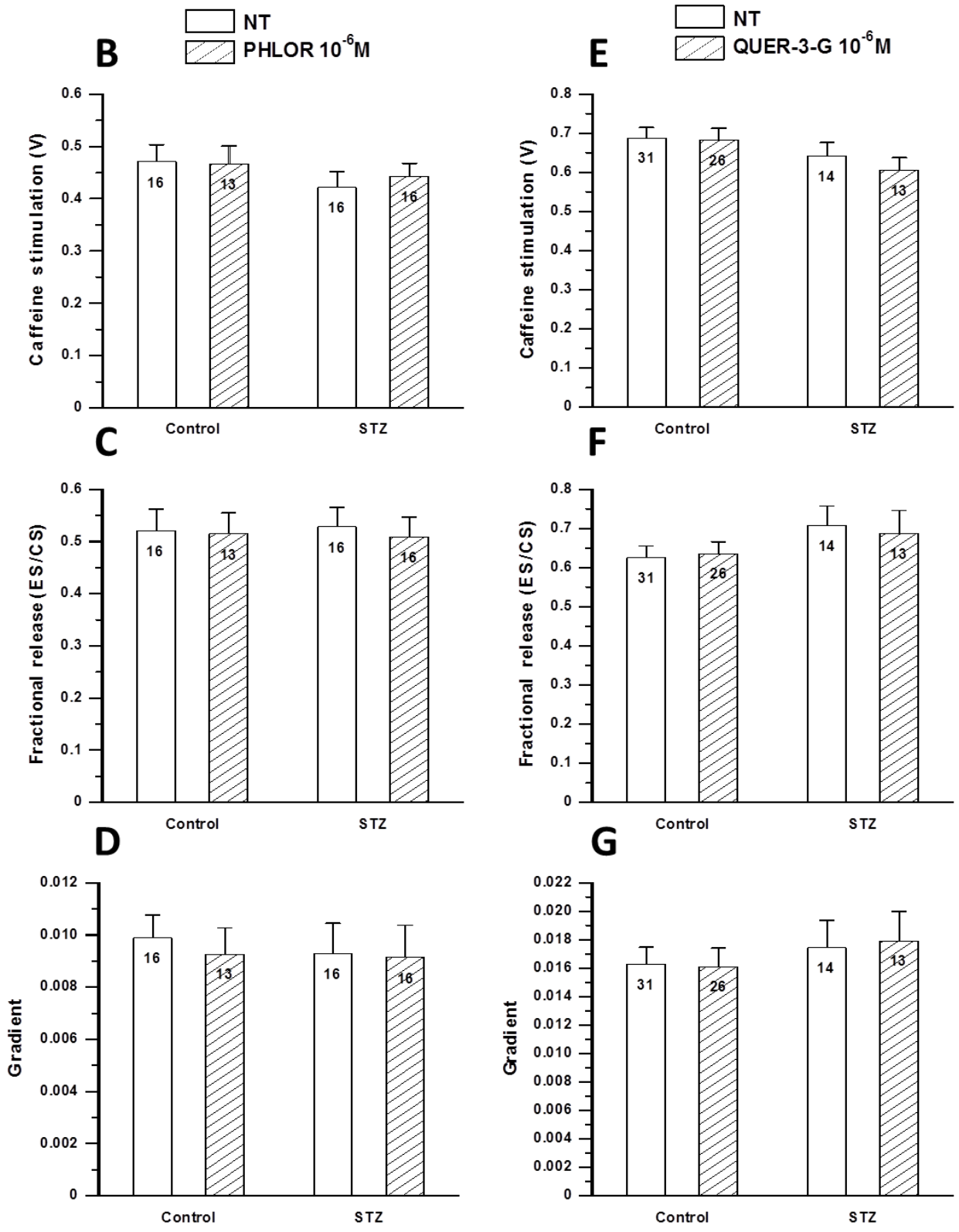
B
NT
PHLOR 10-6M
Caffeine stimulation (V)
0.6
0.5
0.4
0.3
0.2
0.1
0
16
13
16
16
Control
STZ
E
NT
QUER-3-G 10-6M
Caffeine stimulation (V)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
31
26
14
13
Control
STZ
C
Fractional release (ES/CS)
0.6
0.5
0.4
0.3
0.2
0.1
0
16
13
16
16
Control
STZ
F
Fractional release (ES/CS)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
31
26
14
13
Control
STZ
D
Gradient
0.012
0.010
0.008
0.006
0.004
0.002
0
16
13
16
16
Control
STZ
G
Gradient
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0
31
26
14
13
Control
STZ

Figure 4B-G