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Hyperglycemia induces mechanical hyperalgesia and depolarization of the resting membrane potential of primary nociceptive neurons: Role of ATP-sensitive potassium channels

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ABSTRACT

Cumulating data suggests that ion channel alterations in nociceptive neurons might be involved in the development of diabetic painful neuropathy. In the present study we investigated the involvement of ATP-sensitive potassium (K^+_{ATP}) channels in the acute effect of high glucose solution *in vitro* and *in vivo*. High glucose concentrations depolarized cultured nociceptive neurons and depolarization was blocked by the K^+_{ATP} opener, diazoxide or by insulin. Glucose injection at the rat dorsal root ganglia (L5) resulted in acute mechanical hyperalgesia that was blocked by diazoxide. Mannitol injection indicates that osmolarity changes are not responsible for glucose effect. Therefore, this study suggests that K^+_{ATP} channels expressed in peripheral sensory neurons might be involved in the development of diabetic painful neuropathy. Since sulfonylureas, that act by blocking K^+_{ATP} are used for diabetes treatment, it is important to evaluate the possible side effects of such drugs at primary sensory neurons.

1. Introduction

Diabetes is a relevant and growing health problem for most countries, regardless of development status. In 2017 the International Diabetes Federation estimated that about 8.8% of the world population between 20 and 79 years old was living with diabetes. If current trends persist, it is estimated that the number of people with diabetes will exceed 629 million by 2045 [18]. Diabetic neuropathies are the most prevalent chronic complications, affecting 30 to 50% of diabetic patients. They are characterized by signs of dysfunction in peripheral nervous system, with different clinical manifestations [12]. In the sensory nervous system, diabetic neuropathy can manifest as a reduced or increased sensitivity [28]. Patients with diabetic neuropathy usually experience burning sensations and mechanical allodynia [5,38].

Chronic hyperglycemia seems to be the main cause of diabetic neuropathy. The increased glucose, sorbitol and fructose concentrations in the peripheral nerve generate an osmotic edema, reduction in the synthesis of acetylcholine, decomposition of Schwann cells and myelin. All of this leads to changes in afferent and efferent conduction [26]. Moreover, the formation of advanced glycation products by non-enzymatic binding causes a series of impairments in cell function, including in the processes of cell transcription and signaling [4]. While reduced sensations seems to occur due to demyelination and axonal degeneration [11], the mechanism responsible for the increase in somesthetic sensations, such as neuropathic pain, however, is less understood.

It has been suggested that changes in ion channels functions might be responsible for producing sensitivity alterations observed in neuropathic disorders (for review, [24,44]).

In addition to the chronic hyperglycemia effects, which lead to the development of diabetic neuropathy, some studies suggest that both hyperglycemia ([31,40,41]) and insulinemia or insulinopenia [6,32] can act acutely by promoting rapid effects on sensory neurons, probably by altering neuronal excitability. Such direct effects may be involved in the development of painful neuropathy and to comprehend such mechanisms can contribute to minimize the progression of this chronic diabetic complication.

In the present study we tested the acute effect of hyperglycemia at primary sensory neurons *in vivo,* through local administration of glucose at the rat dorsal root ganglia, and also *in vitro,* through resting membrane potential evaluation using a fluorescent probe in primary dorsal root ganglia cultures.

The ATP-sensitive potassium channel (K^+_{ATP}) is a Kir subunit coupled

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with a sulfonylurea and is essential for glucose-induced insulin release by pancreatic β-cells. In these cells, intracellular ATP concentration is increased by glucose metabolism causing the K^+_{ATP} channels to close, depolarizing cell membrane [3,37].

Previous studies by Cunha et al. [8,9] show that K^+_{ATP} channels on nociceptive neurons are involved in the peripheral analgesia induced by opioids and dipyrone [1]. In this case, we observed that opening of K^+_{ATP} channels by the analgesic drugs induces resting membrane potential hyperpolarization. Furthermore, peripheral sensory neurons are permeable to glucose and express the glucokinase enzyme [14]. Therefore, in the present study it was tested if acute glucose administration affects nociceptive neurons through K^+_{ATP} channel modulation. We also tested the effect of high glucose administration at rat dorsal root ganglia and the involvement of K^+_{ATP} channels on mechanical hyperalgesia.

2. Material and methods

2.1. Animals

Male Wistar rats (200-250 g) were housed with food and water *ad libitum* in a controlled environment of 12/12 h light/dark cycle. All experiments were approved by the Committee for Ethical Use of Animals from Universidade Federal de Uberlândia (CEUA/UFU n 050/2017).

2.2. Primary cell culture of dorsal root ganglion

Rats were euthanized under isoflurane anesthesia and lumbar and thoracic DRGs were harvested and transferred to Hank's buffered saline solution containing Hepes (10 mM). Ganglia were incubated in 0.28 U/mL collagenase (type II; Sigma) for 75 min and in 0.25% trypsin (Sigma) for 12 min. After three washes with DMEM containing 10% of fetal bovine serum, the ganglionic cells were dissociated using a fire-polished glass Pasteur pipette and plated in glass bottom dishes coated with Matrigel (BD). Cultures were maintained in DMEM plus 10% of fetal bovine serum and penicillin (50 U/mL)/streptomycin (50 mg/mL) for 24 h at 37 °C with 5% CO2 atmosphere.

2.3. Drugs

The agents used in this study were obtained as follows: $DiBAC_4(3)$ (Invitrogen), glibenclamide (Sigma), diazoxide (Sigma), glucose (Êxodo), insulin (Sigma), capsaicin (Sigma), mannitol (Synth) and dimetil sulfoxide - DMSO (Vetec).

For the cell cultures $DiBAC_4(3)$ [5 μM], capsaicin, insulin, and glucose were diluted in a Hank's/Hepes solution; diazoxide and glibenclamide were diluted in Hank's/Hepes from a stock solution in DMSO. The highest DMSO concentration administered was 1%. In one test, glucose was diluted in water in order to adjust the osmolarity of the solution. All drugs were applied at primary cultures by pipetting 10× the desired final concentration, diluted at the plates during image acquisition.

For *in vivo* experiments, glucose and mannitol were diluted in saline [0.9%], the vehicle. Diazoxide was diluted in saline [0,9%] from a stock solution in DMSO. Glucose was also diluted in 1% DMSO for control.

2.4. Confocal microscopy

Cultures were washed with Hanks/Hepes buffered solution and incubated for 30 min with 5 μM of $DiBAC_4(3)$ also diluted in Hanks/Hepes at room temperature in the dark. The fluorescent probe remained in the culture dishes during experiments as the molecule movement across the plasma membrane, according to its electrochemical gradient, determines fluorescence variations. As $DiBAC_4(3)$ is negatively charged, an increase in intracellular fluorescence indicates membrane depolarization and *vice-versa*. Serial images were acquired using a confocal microscope (LSM 510 meta, Zeiss). Images were acquired during 10 min at 3 s intervals. After acquisition of basal fluorescence during serial image acquisition, drugs were administered using an automatic pipette by adding 10 μL of 10× the final concentration of the drug to 90 μL of the Hanks solution bathing the cultured cells. After each test, capsaicin (1 μM) was applied and fluorescent images registered during 2 min. Only neurons that responded to capsaicin with membrane depolarization were selected for analysis. Capsaicin sensitivity allowed the selection of nociceptive neurons that were alive and functional in the primary cultures.

Images were analyzed using Image J software (developed by Wayne Rasband, NIH, USA). Regions of interest (ROIs) containing individual neurons were selected and fluorescence intensities quantified at the image series. For determination of fluorescence variation data were calculated as $(\Delta F/F_0)$, in which $\Delta F = F\text{-}F_0$ and F_0 is the fluorescence intensity at basal conditions, before drug administration. Each experiment was repeated in 3 different plates, obtained from at least two animals, and individual cell responses were considered for statistics.

2.5. Ganglionar drug administration

In order to test the effects of hyperglycemia and diazoxide in mechanical sensitivity we used a direct intraganglionar (i.gl.) injection, as described elsewhere [13]. The drugs or their respective vehicles were administered in the right L5 dorsal root ganglia (5 μL volume). Different animals were used in each experimental group.

2.6. Evaluation of mechanical sensitivity

The mechanical nociceptive threshold was measured by the electronic von Frey method as previously described [42]. In a quiet room, rats were placed in acrylic cages (12 × 20 × 17 cm) with wire grid floors, 15–30 min before the start of testing. During this adaptation period, the paws were tested (probed) three times. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer adapted with a 0.7-mm^2 polypropylene tip. A tilted mirror placed under the grid provided a clear view of the rat hind paw. The investigator was trained to apply the tip in between the five distal footpads with a gradual increase in pressure. The stimulus was automatically discontinued and its intensity was recorded when the paw was withdrawn. The endpoint was characterized by the removal of the paw in a clear flinch response after the paw withdrawal. The animals were tested before and after treatments. Different investigators performed the test and administered the ganglionar injections.

2.7. Statistical analysis

Behavioral and *in vitro* fluorescence data are presented as mean ± SEM and compared by one-way or two-way ANOVA followed by Bonferroni tests using GraphPad Prism software. Means were considered significantly different if $P < 0.05$.

3. Results

3.1. Glucose-induced alteration on the membrane potential of dorsal root ganglia neurons

Primary cultures of dorsal root ganglia were incubated with 5 μM of $DiBAC_4(3)$ during 30 min and then transferred to confocal microscope for serial image acquisition. Glucose (16, 25 or 33 mmol/L) or vehicle was administered immediately after the first 2–3 images and the fluorescent images acquired during 10 min (Fig. 1). After each experiment, capsaicin (1 μM) was administered and images acquired during an additional 2 min. Only neurons that responded to capsaicin with membrane depolarization were considered for analysis.

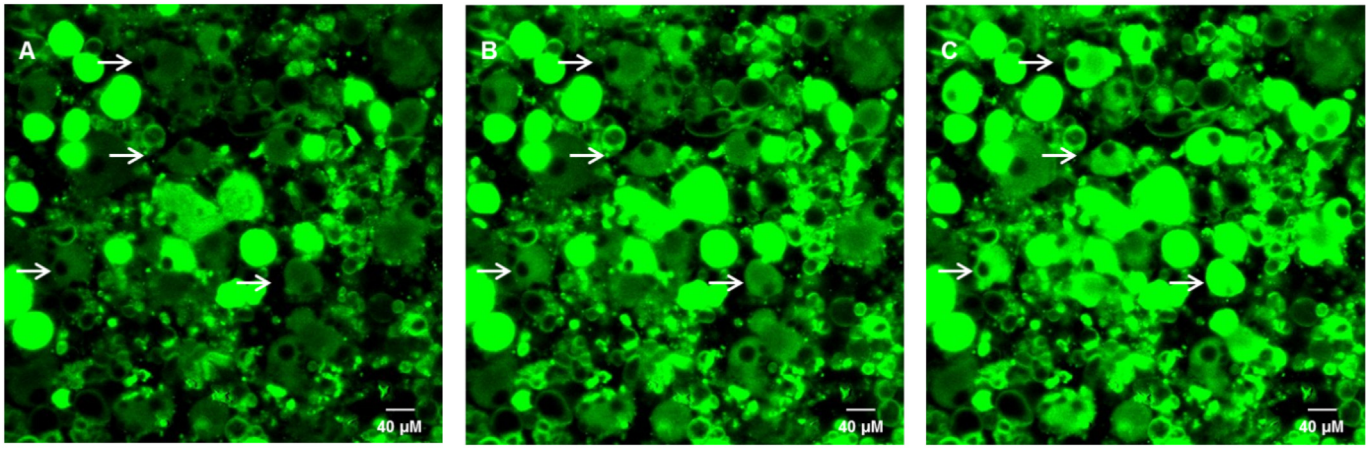

Fig. 1. Confocal fluorescent images of primary cultures of dorsal root ganglia in the presence of the resting membrane potential probe $DiBAC_4(3)$. A: Basal fluorescence. B: At 10 min after glucose (33.3 mmol/L) administration. C: At 2 min after capsaicin administration. Arrows indicate some of the neurons that responded to glucose and capsaicin. Images obtained using 40× magnification.

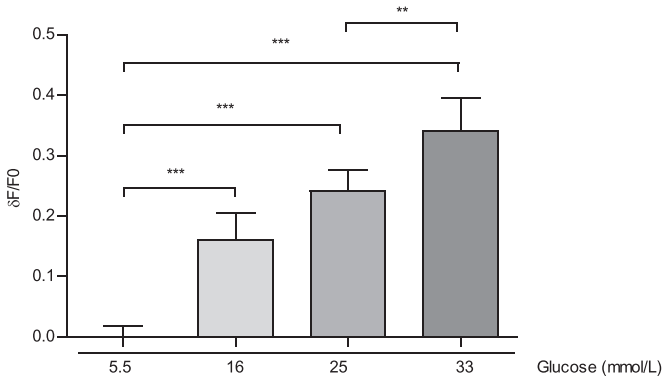

Fig. 2. Glucose effect on resting membrane potential of cultured nociceptive neurons. The variation of fluorescence intensities emitted by DiBAC4(3) after 10 min of image acquisition of dorsal root ganglia cultures in Hank's solution (5.5 mmol/L glucose) or after administering glucose to a final concentration of 16, 25 or 33 mmol/L are shown. Only neurons that responded to capsaicin (1 μM) were considered for analysis. Data shown as means ± S.E.M of 31 to 125 neurons. ***$p < 0{,}0001$; **$p < 0{,}001$. (ANOVA followed by Bonferroni posttest).

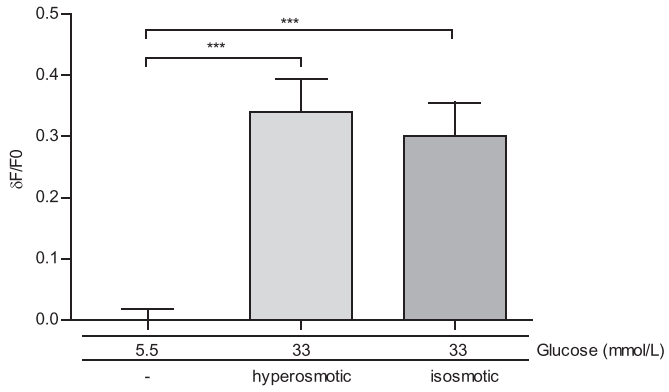

Fig. 3. Depolarization induced by glucose on primary sensory neurons. Figure shows the variation of fluorescence intensities emitted by DiBAC4(3) after 10 min of image acquisition of dorsal root ganglia cultures in Hank's solution (5.5 mmol/L glucose) or after administering glucose to a final concentration of 33 mmol/L (diluted in Hank's or H_2O). Only neurons that responded to capsaicin (1 μM) were considered for analysis. Data shown as means ± S.E.M of 34 to 125 neurons. ***$p < 0{,}0001$. (ANOVA followed by Bonferroni posttest).

Hyperglycemia induced neuronal depolarization in capsaicin-sensitive nociceptive neurons in a concentration-dependent manner (Fig. 2). All glucose concentrations tested caused an increase in DIBAC4(3) fluorescence. The fluorescence changes induced by hyperglycemia were: 16 mmol/L glucose (0.1605 ± 0.04496; $n = 60$); 25 mmol/L glucose (0.2420 ± 0.03510; $n = 31$) and 33 mmol/L (0.3405 ± 0.05428; $n = 34$). Hank's administration (5.5 mmol/L glucose) did not induce fluorescence alterations (0.0005373 ± 0.01768; $n = 125$).

Experiments were performed by increasing the amount of glucose to the 5.5 mmol/L concentration present in Hank's buffer. For this purpose, glucose was added in a 10× concentration in Hanks solution and diluted to the final concentration in the culture dish during image acquisition. Therefore, as the glucose was added, the osmolarity of the solution was slightly increased. At the highest glucose concentration tested, 33 mmol/L, the administration of glucose caused an increase of 28 mOsm in the solution bathing the cells. As changes in osmolarity might affect cell function, we tested the administration of 33 mmol/L glucose in an isosmotic solution. This was achieved by solubilizing the 10× concentration of glucose (27 mmol/L) in water (Fig. 3) and then diluting to the final concentration in Hank's at the culture dish. The osmolarity of 27 mmol/L glucose is 280 mOsm, which is compatible with the osmolarity of the Hank's solution.

The results show that glucose depolarization is caused by glucose itself and not due to osmolarity alteration since there was no significant difference between the administration of glucose diluted in Hank's (0.3405 ± 0.05428; $n = 34$) and glucose diluted in water (0.3003 ± 0.05521; $n = 41$) comparing to the vehicle Hank's (5.5 mmol/L of glucose) administration (0.0005373 ± 0.01768, $n = 125$).

3.2. Involvement of K^+_{ATP} channels on the depolarization induced by glucose on primary sensory neurons

To test for the involvement of K^+_{ATP} channels, glucose (25 or 33 mmol/L) was administered in the presence of diazoxide (10 μM), a drug that selectively opens K^+_{ATP} channels. Diazoxide inhibited glucose induced neuronal depolarization (Fig. 4). Treatment with diazoxide (25 mmol/L: 0.003080 ± 0.01958; $n = 20$) (33 mmol/L: 0.1552 ± 0.07947; n = 20), decreased cell depolarization, compared to depolarization caused by glucose (25 mmol/L) (0.2420 ± 0.03510; $n = 31$) or high glucose (33 mmol/L) (0.1605 ± 0.04496; $n = 60$) concentrations. Hank's solution, the vehicle (5.5 mmol/L glucose) administration did not induce fluorescence alterations (0.0005373 ± 0.01768; $n = 125$).

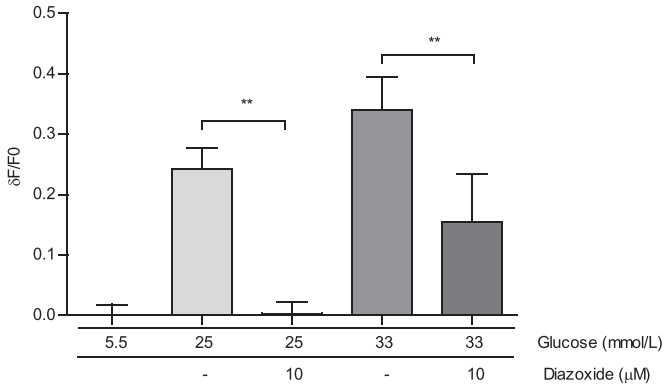

Fig. 4. Effect of glucose on cell depolarization in the presence of the K^+_{ATP} channel opener diazoxide. Figure shows the variation of fluorescence intensities emitted by DiBAC4(3) after 10 min of image acquisition of dorsal root ganglia cultures in Hank's solution (5.5 mmol/L glucose) or after administering glucose (25 or 33 mmol/L) or glucose plus diazoxide (10 μM). Only neurons that responded to capsaicin (1 μM) were considered for analysis. Data shown as means ± S.E.M of 20 to 125 neurons. $^{**}p < .05$. (ANOVA followed by Bonferroni posttest).

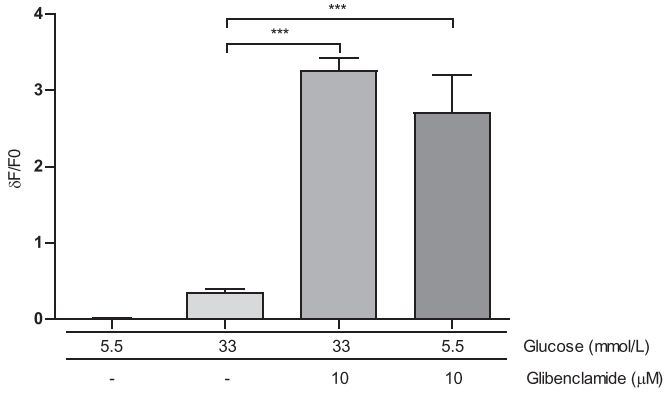

Fig. 5. Effect of the K^+_{ATP} channel inhibitor on neuronal resting membrane potential. Figure shows the variation of fluorescence intensities emitted by DiBAC4(3) after 10 min of image acquisition of dorsal root ganglia cultures in Hank's solution (5.5 mmol/L glucose) or after administering glucose (33 mmol/L) or glucose plus glibenclamide (10 μM) or glibenclamide itself (10 μM). Only neurons that responded to capsaicin (1 μM) were considered for analysis. Data shown as means ± S.E.M of 19 to 133 neurons. $^{***}p < .001$. (ANOVA followed by Bonferroni posttest).

The effect of glibenclamide 10 μM, a K^+_{ATP} channel inhibitor, was evaluated to determine the role of K^+_{ATP} channels on neuronal resting membrane potential (Fig. 5). Glibenclamide (10 μM) administration *per se* (2.709 ± 0.4845; $n = 18$) or in the presence of high glucose concentration (33 mmol/L) (3.254 ± 0.1713; $n = 30$) resulted in strong depolarization, as observed by the increase in fluorescence emitted by DiBAC4(3), compared to glucose (33 mmol/L) by itself (0.1605 ± 0.04496; $n = 60$) and with Hank's (0.0005373 ± 0.01768; $n = 125$).

3.3. Insulin administration inhibits glucose-induced cell depolarization

As diabetes-induced hyperglycemia is caused by impaired insulin release or action, it was decided to test whether insulin interferes with depolarization induced by hyperglycemia in nociceptive neurons. With this aim, dorsal root ganglia cultures were treated with glucose (33 mmol/L) in the presence of insulin (1 μM) (Fig. 6). Insulin administration caused a significant reduction of neuronal depolarization (0.1351 ± 0.04079; $n = 14$), induced by 33 mmol/L glucose

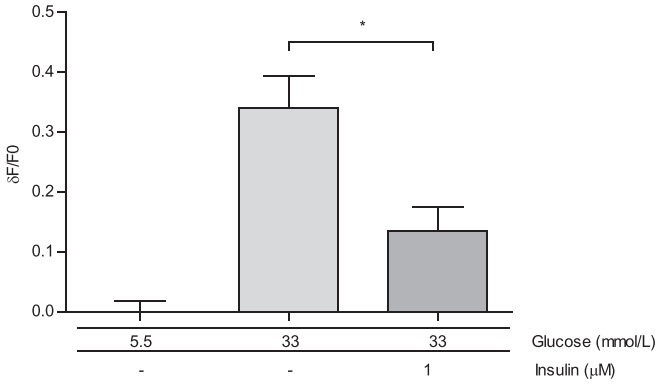

Fig. 6. Insulin inhibits the glucose-induced cell depolarization. Figure shows the variation of fluorescence intensities emitted by DiBAC4(3) after 10 min of image acquisition of dorsal root ganglia cultures in Hank's solution (5.5 mmol/L glucose) or glucose (33 mmol/L) or glucose plus insulin (1 μM). Only neurons that responded to capsaicin (1 μM) were considered for analysis. Data shown as means ± S.E.M of 14 to 133 neurons. $^{*}p < .05$. (ANOVA followed by Bonferroni posttest).

(0.1605 ± 0.04496; $n = 60$).

3.4. Evaluation of acute glucose administration at rat dorsal root ganglia on mechanical sensitivity

To test if hyperglycemia might affect dorsal root ganglia neurons *in vivo,* the thresholds for mechanical stimulation of the hind paw were evaluated in rats treated with injections at the right L5 dorsal root ganglia. Mechanical thresholds of the right hind paw were obtained using the electronic von Frey test. Fig. 7 shows the acute effect of intraganglionar (i.gl.) injections of glucose (16, 25 or 33 mmol/L) or vehicle (saline) on mechanical sensitivity. We also tested the effect of mannitol injection (33 mmol/L) in order to investigate a possible effect of osmolarity increase at the dorsal root ganglia.

The threshold of mechanical sensitivity was reduced in the animals treated with glucose at the concentration 25 mmol/L 15 (56.41) and 30 (58.57) minutes after intraganglionar injection and at the concentration 33 mmol/L 15 (40.54), 30 (45.78) and 60 (57.89) minutes after injection, compared to the control group (Basal 74.52; 15 min 71.10; 30 min 73.49; 60 min 72.63).

Glucose at concentration 16 mmol/L (Basal 73.84; 15 min 72.18; 30 min 70.04; 60 min 69.49) did not alter the threshold of mechanical sensitivity. This also happened with mannitol (Basal 68.54; 15 min 71.69; 30 min 72.13; 60 min 71.02), showing that the change is not due to osmolarity.

3.5. K^+_{ATP} channel opener blocks the increase in glucose-induced sensitivity

The participation of K^+_{ATP} channels at the mechanical hyperalgesia induced by glucose *in vivo* was tested through administration of the selective opener diazoxide together with glucose at rat dorsal root ganglia (Fig. 8).

These results show that the potassium channel opener diazoxide does not allow for a decrease in the threshold of mechanical sensitivity induced by glucose (Basal 69.83; 15 min 70.68; 30 min 73.47; 60 min 71.05) as occurs in control groups vehicle (Basal 74.52; 15 min 71.10; 30 min 73.49; 60 min 72.63) and diazoxide (Basal 69.04; 15 min 69.59; 30 min 71.66; 60 min 71.15). Only in the group in wich glucose was given alone was there a decrease in the threshold (Basal 71.71; 15 min 53.65; 30 min 57.09; 60 min 60.98) at 15, 30 and 60 min after intraganglionar injection.

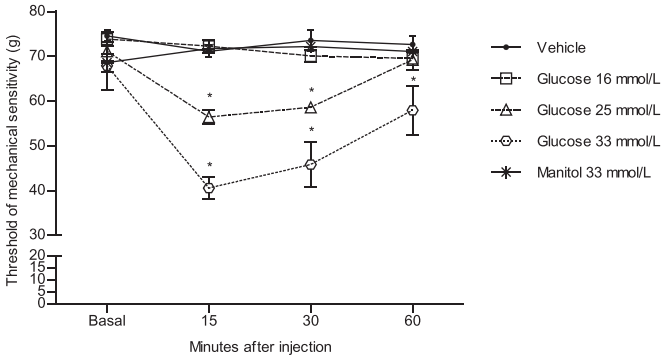

Fig. 7. Glucose concentration dependent effect on the threshold of mechanical sensitivity. Figure shows the threshold of mechanical sensitivity (g) of glucose (16, 25 or 33 mmol/L), mannitol (33 mmol/L) or vehicle (saline) administration at the dorsal root ganglia (right L5, 5 μL). Data shown as means ± S.E.M. 6 animals per group. *$p < .05$. (ANOVA followed by Bonferroni posttest).

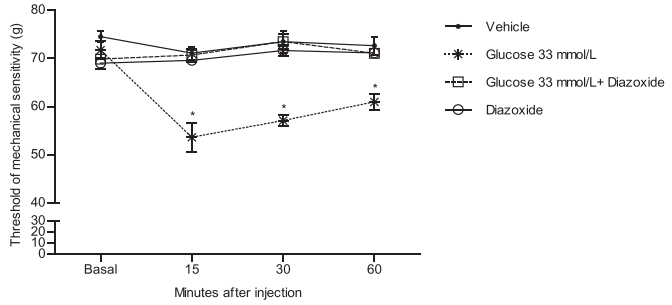

Fig. 8. Effect of glucose on threshold of mechanical sensitivity in the presence of K^+_{ATP} channel opener. Figure shows the threshold of mechanical sensitivity (g) of glucose (33 mmol/L), glucose (33 mmol/L) plus diazoxide (10 μM), diazoxide (10 μM) or vehicle (1% DMSO in saline). Data shown as means ± S.E.M. 6 animals per group. *$p < .05$. (ANOVA followed by Bonferroni posttest).

4. Discussion

This work evaluated the effect of hyperglycemia on rat mechanical hyperalgesia and on the resting membrane potential of cultured nociceptive neurons. Present findings provide support for the idea that hyperglycemia has an acute effect on nociceptive neurons that contribute to a state of hyperalgesia, as other studies also suggest ([21,22,23]). Furthermore, results herein described indicate that K^+_{ATP} potassium channels are involved in the resting membrane depolarization induced by hyperglycemia and also in the *in vivo* hyperalgesia.

Using the fluorescent probe, DiBAC4(3), to detect changes in resting membrane potential of cultured primary sensory neurons we found that increasing glucose concentration led to membrane depolarization. As neurons were selected according to capsaicin sensitivity, membrane potential alterations were detected in nociceptive neurons associated with C fibers. A similar result was described from gastric-projecting neurons obtained from nodose ganglia [15], which had a significantly high excitatory response associated with membrane potential depolarization under hyperglycemia conditions. Also, it was previously shown that hyperglycemia can act directly on inhibitory motor neurons that mediate gastric inhibitory vagovagal circuits and in smooth muscle cells, where hyperglycemia causes depolarization and increased excitability [16].

In the *in vitro* experiments to test for hyperglycemia effect on nociceptive neurons, 10× glucose final concentration was diluted in Hank's and added to the solution bathing the cells. Therefore, the augmented glucose concentration resulted in an increased osmolarity of the solution. Matsuka and Spigelman [25] have found that hyperosmolar solutions (containing increased glucose, sucrose, NaCl, or mannitol) produce a selective block of signal propagation in myelinated sensory A-fibers. To test if changes were responsible for neuronal resting membrane potential alterations, we tested the administration of an isosmotic solution of 33 mmol/L glucose. Isosmotic solution with 33 mmol/L glucose did not change the depolarization induced by the same concentration of glucose in the hyperosmotic solution. Therefore, the effect observed was not caused by the osmotic pressure variation.

Membrane resting potential is regulated by ion channels and ion pumps. High glucose concentration was associated with a reduced activity of the Na^+,K^+-ATPase in several tissues [36], which might result in membrane depolarization. Glucose induced neuronal depolarization has been previously described in afferent neurons from the nodose ganglia involved in controlling gastric motility [43]. In this study it was found that K^+_{ATP} channels are involved in neuronal depolarization and gastric relaxation induced by glucose.

K^+_{ATP} channel is a hetero-octameric complex of four pore-forming Kir6.x subunits and four regulatory sulfonylurea receptor (SURx) subunits, and it conducts a potassium current that is inhibited by ATP binding to Kir6.x subunits and stimulated by ADP interaction with sites within SURx subunits [17]. These channels are essential for glucose induced insulin release by pancreatic β cells. Peripheral sensory neurons are permeable to glucose [14] and express K^+_{ATP} channels [29,30]. Threfore, a similar mechanism as the one responsible for glucose induced insulin release by pancreatic β cells could explain hyperglycemia induced depolarization in nociceptive neurons. To test for K^+_{ATP} channels involvement, we investigated the effects of pharmacological activation and inactivation of K^+_{ATP} channels on resting membrane potential of primary sensory neurons. Diazoxide and glibenclamide were used as a K^+_{ATP} channel activator and inactivator, respectively. The administration of diazoxide blocked the depolarization induced by hyperglycemia on nociceptive neurons, suggesting the involvement of K^+_{ATP} channels. Furthermore, we observed that glibenclamide, a K^+_{ATP} channel inhibitor, is able to depolarize the nociceptive neurons. Therefore, open K^+_{ATP} channels seem to participate in potassium currents during resting conditions.

It has already been shown that K^+_{ATP} channels, expressed in nociceptive neurons, play a crucial role on mechanical hyperalgesia. The peripheral antinociceptive actions of opioids, diclofenac and dypirone are dependent on K^+_{ATP} channels opening ([1,2,8]; [35]). The activation of PI3K/AKT signaling pathway by opioids stimulate the production of nitric oxide and it promotes the opening of K^+_{ATP} channels. This pathway might cause a hyperpolarization of nociceptive neurons counteracting their enhanced excitability in an inflammatory process [8,9]. It is interesting to note that insulin receptor activation also led to PI3K/AKT activation in primary sensory neurons [20]. Therefore, it is possible that the insulin effect on nociceptive neurons result in K^+_{ATP} channels opening, thus counteracting the glucose effect. Insulin effect on K^+_{ATP} channels might explain the decreased mechanical and thermal nociceptive threshold observed in animals with reduced insulin production [31,32]. This interesting hypothesis remains to be tested.

The results obtained with *in vitro* administration of high glucose concentrations suggested that glucose might have a direct acute effect on nociceptive sensitivity. A direct effect of hyperglycemia was described in a study that tested the effect of chronic administration of glucose solutions at the dorsal root ganglia using osmotic pumps [10].

In this study, an increased mechanical sensitivity in animals, that started soon after treatment initation, was detected. To test for an acute effect of hyperglycemia and the involvement of K + ATP channels, we used direct injections in the rat right L5 dorsal root ganglia [13]. Results show that glucose injection in the dorsal root ganglia acutelly increase mechanical sensitivity. The effect was observed from 15 to 30 min and was probably not sustained because of glucose metabolization by the cells. As observed *in vitro*, the effect was not due to ormolarity increase, as mannitol at the same concentration of the highest glucose concentration tested (33 mmol/L) did not affect mechanical threshold. The hyperglycemia effect on mechanical sensitiviy was blocked by co-administration of the K^+_{ATP} channel openner diazoxide, suggesting that K^+_{ATP} channels are involved in acute mechanical hyperalgesia induced by glucose at the dorsal root ganglia.

There is a strong association between hyperglycemia and neuropathy, because diabetic neuropathy can be prevented or even slowed when glucose levels are controlled [7] The role of β-cell K^+_{ATP} channels in insulin secretion has been well-characterized and sulfonylureas like glibenclamide are widely used for the treatment of type-2 diabetes and K^+_{ATP} channels closure results in depolarization of the β-cell membrane and stimulation of insulin secretion [33,39]. However, as we observed that K^+_{ATP} channels might be involved in a direct glucose effect on primary sensory neurons, it would be important to consider such effect on sulfonylurea prescription. In fact, there is evidence that diabetic patients undergoing sulfonylurea treatment might present a higher risk for neuropathy development [19,27]. Further studies are necessary to investigate such a relationship between sulfonylurea treatment and diabetic neuropathy.

5. Conclusion

Taken together, data described in the present study suggests that K^+_{ATP} channels are involved in an acute effect of hyperglycemia at nociceptive neurons. Studying the involvement of K^+_{ATP} channels in the development of chronic diabetic sensory neuropathy might provide valuable information for prevention of this condition. Furthermore, as sulfonylureas are commonly used in diabetes treatment, a possible side effect of such drugs at primary sensory neurons should be investigated.

Conflicts of interest

The authors declare no conflicts of interest.

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