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Effect of heat stress and Hsp90 inhibition on T-type calcium currents and voltage-dependent potassium currents in leydig cells

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ABSTRACT

Heat can trigger testicular damage and impair fertility. Leydig cells produce testosterone in response to stimulation by luteinizing hormone (LH), which induces Ca^{2+} entry and K^+ efflux through ion channels in their plasma membrane. Considering that mechanisms coordinating the Leydig cell responses to hyperthermic stress remain unclear; the present study analyzed the effects of heat stress (HS, 43°C, 15 min) and inhibition of Hsp90 on T-type calcium currents and voltage-dependent potassium currents (VKC) in mice Leydig cells. Results show that HS reduced the VKC steady state currents at +80 mV (45.3%) and maximum conductance (71.5%), as well as increased the activation time constant (31.7%) and the voltage for which half the channels are open (30%). Hsp90 inhibition did not change the VKC currents. T-type calcium currents were not affected by HS or Hsp90 inhibition. In conclusion, HS can slow the activation, reduce the currents and voltage dependence of the VKC, suggesting a possible role of these currents in the response to hyperthermic stress in Leydig cells.

1. Introduction

Approximately 8% of men have fertility problems, of whom 10% had reversible factors affecting their fertility potential (Esteves et al., 2011). Heat is an agent that can trigger testicular damage and impairment of their normal function (Ayaz et al., 2018). In principle all tissues are susceptible to damage by heat. However, testes are particularly sensitive and are damaged by exposure to temperatures typically found inside the abdomen (cryptorchidism), their normal function occurs between 2 and 6 °C below body temperature (Jensen et al., 2006; Oka et al., 2017). Despite the fact that germ cells show great sensitivity to heat, Leydig cells are more resistant (Setchell, 2006); however, there are reports of changes in Leydig cells exposed to heat (Kim et al., 2016). Research on thermal stress has focused on the impact of heat on spermatogenesis, with rather little attention paid to the molecular effects of heat treatment on Leydig cell function (Li et al., 2015).

Spermatogenesis in adulthood is regulated by a complex and interconnected network of endocrine, paracrine and autocrine interactions. Testosterone is one of the main factors regulating spermatogenesis, which in adults is essential for the regulation of sexual behavior, functional maintenance of spermatogenesis, epididymis and accessory sex glands (Holdcraft and Braun, 2004; Oka et al., 2017). Leydig cells produce testosterone in response to the binding of luteinizing hormone (LH) to its 7-transmembrane G-protein coupled receptor, resulting in an increase of cAMP and intracellular Ca^{2+} concentration (Svechnikov et al., 2010; Abdou et al., 2016). This increase in intracellular Ca^{2+} concentration is triggered by Ca^{2+} entry through T-type channels located in the plasma membrane (Costa et al., 2010). In response to Ca^{2+} entry, Ca^{2+}-activated K^+ channels (BK_{Ca}) can be activated, inducing K^+ currents in order to repolarize the membrane (Carnio and Varanda, 1995). Both calcium and potassium channels are important for the normal production of testosterone, since inhibition of T-type calcium

Abbreviations: BK_{Ca}, Ca^{2+}-activated K^+ channels; Er, estimated reversal potential; GA, Geldanamycin; G_{max}, maximum conductance; G_{min}, minimum conductance; HS, heat stress; Hsp90, 90 kDa heat shock protein; LH, luteinizing hormone; τ_{act}, time constants of activation; τ_{inact}, time constants of inactivation; V_0, voltage at which half the channels are open; VKC, voltage-dependent potassium currents

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and BK_{Ca} potassium channels reduce the production of testosterone in Leydig cells (Costa and Varanda, 2007; Matzkin et al., 2013).

Exposure to toxic agents may lead to cellular stress and change the cellular homeostasis by several mechanisms [Herr and Debatin, 2001]. Despite the pathophysiological importance of this process, the signaling mechanisms in Leydig cells related to cellular stress are still unclear. Cellular responses to heat stress can change ion currents (Wu et al., 2001; Chen et al., 2010). Many factors are associated with cellular stress, including the 90 kDa heat shock protein (Hsp90). Hsp90 is a molecular chaperone associated with many functions in normal cells and after cellular stress (Zuehlke and Johnson, 2010). Studies have also shown that Hsp's can interact with ion channels (Yan et al., 2010; Walker et al., 2010).

Considering that mechanisms coordinating the Leydig cell responses to hyperthermic stress remain unclear; the present study analyzed the effects of heat stress (HS) and the inhibition of Hsp90 on the activities of T-type calcium currents and voltage-dependent potassium currents (VKC) in Leydig cells.

2. Materials and methods

Freshly isolated Leydig cells were obtained from male Swiss mice (*Mus musculus* - 45–60 days old), according to the methods described by de Deus et al. (2018). In the present study we consider 2 periods of analysis for the Hsp90 inhibitor: 1) short term exposure refers to analysis immediately after exposure to the Hsp90 inhibitor. In all short term experiments the control records of ionic currents were obtained and then cells were treated. These cells were controls of themselves; 2) long term exposure refers to analysis 2 h after exposure to the Hsp90 inhibitor, ionic currents from different cells were compared between these groups.

All heat stress experiments (43 ° C) were performed 2 h after exposure to this high temperature, at which time the solution with the cells had already returned to room temperature (23 ± 2 °C), avoiding direct effect of the temperature in the analyzes.

Leydig cells were collected and submitted to the following conditions (Table 1):

Hsp90 blockage was done with 17–Dimethylaminopropylamino–17–demethoxy geldanamycin (17-DMAP-GA) (Tian et al., 2004) obtained from Invivogen (San Diego, USA) at a concentration of 10 μM.

The experimental protocols were approved by the Ethics Committee for Animal Use in Research of the Federal Rural University of Pernambuco (UFRPE n. 23082022244710) in accordance with the basic principles for research using animals.

2.1. Temperature control during heat stress

Solutions containing the Leydig cells were warmed with a water bath (Cienlab CE-160; Campinas, Brazil) at 43° C for 15 min (Chen et al., 2010). Room temperature was 23 ± 2 °C. Temperature was controlled using a thermometer accurate to 0.1 °C (Incoterm 7665; Porto Alegre, Brazil).

2.2. Electrophysiology

Experiments were performed using the patch clamp technique in the whole-cell configuration. Currents were measured with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, USA). Ionic currents were low pass filtered at 2 kHz (4-pole Bessel Filter), sampled at 5 kHz and fed to a computer through a Digidata 1440A board (Molecular Devices, Sunnyvale, USA), controlled by the software WINWCP v.4.3.3 (Strathclyde Software, Glasgow, UK). This software was also used to generate and apply the voltage protocols. Patch pipettes with resistance ranging from 2 to 5 MΩ were pulled from borosilicate glass capillaries (Sutter Instrument, Novato, USA) using a P-97 horizontal puller (Sutter Instrument, Novato, USA).

Table 1
Experimental protocols for exposure to heat stress (HS) and treatment with geldanamycin (GA) to inhibit Hsp90.

	Control	GA	HS	HSGA
Short term exposure to GA.	Ion current recording 2 h after cell isolation. Cells were controls of themselves.	Ion current recording soon after addition of Geldanamycin in cells isolated 2 h before. Cells were controls of themselves.	Ion current recording 2 h after heat stress. Cells were controls of themselves.	Ion current recording soon after addition of Geldanamycin in cells exposed to heat stress 2 h before.
Long term exposure to GA.	Ion current recording 2 h after cell isolation.	Ion current recording 2 h after addition of Geldanamycin.	Ion current recording 2 h after heat stress.	Ion current recording after 2 h of exposure to HS and Geldanamycin.

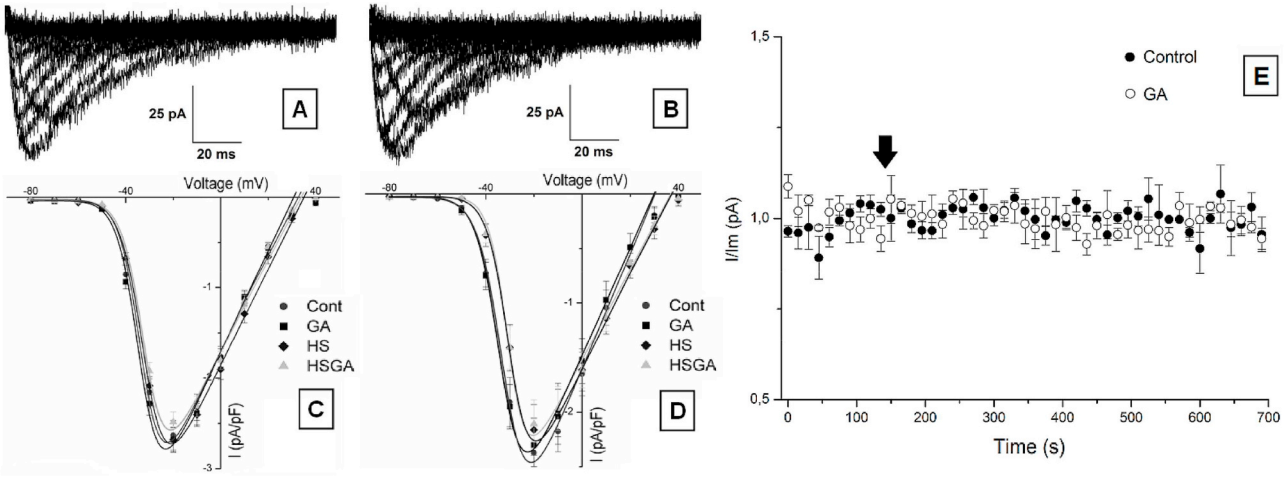

Fig. 1. T-type Ca^{2+} currents in mice Leydig cells elicited with voltage steps between -80 mV and +40 mV from a holding -40 mV. 1A: Raw traces of Ca^{2+} currents in control cells. 1B: Ca^{2+} currents 2 h after exposure to heat stress (HS) and geldanamycin (GA, Hsp90 inhibitor). 1C: I-V relationships of control cells (Cont) or cells exposed to HS and/or GA for 2 h (n_{Cont} = 22, n_{GA} = 23, n_{HS} = 20, n_{HSGA} = 19 cells). 1D: I-V relationships in cells treated with GA shortly after the control records (n_{Cont} = 10, n_{GA} = 10, n_{HS} = 10, n_{HSGA} = 10 cells). 1E: Peak currents elicited by voltage pulses to -20 mV, every 15 s, from a holding -40 mV. Arrow indicates the time of addition of GA to the bathing solution (n = 6). Values were normalized by the average of the points obtained in control conditions.

2.3. Ca^{2+} currents

Ca^{2+} currents were measured in response to voltage steps between -80 mV and +40 mV (10 mV increments) for 200 ms, every 3 s, from a holding potential -80 mV.

Peak currents, normalized by membrane capacitance, were plotted against voltage (I-V curve) and fitted by a Boltzmann function (Costa and Varanda, 2007):

$$I = \left[G_{min} + \frac{(G_{min} - G_{max})}{(1 + \exp((V - V_0)/k))} \right](V - Er) \quad (1)$$

where: I = peak current for each voltage; G_{min} = minimum conductance; G_{max} = maximum conductance; V = membrane voltage; V_0 = voltage at which half the channels are open; k = factor related to the voltage dependence of the system; Er = estimated reversal potential. All I-V curves were fitted with at least $R^2_{mean} = 0.99$.

Time constants of activation (τ_{act}) and inactivation (τ_{inact}) of the Ca^{2+} currents were obtained using voltage steps to -20 mV from a holding potential -40 mV and adjusting a single exponential function describing the current curve (Costa and Varanda, 2007):

$$I_t = \sum_{i=1}^{n} A_i e^{-t/\tau_i} + I_{max} \quad (2)$$

where: I_t = current at time t; A_i = adjustable parameter of the exponential function that describe the current I_t; t = time; τ_i = time constant; I_{max} = maximum current.

The external solution bathing the Leydig cells contained (mM): 126 NaCl, 5 KCl, 10 $CaCl_2$, 1 $MgCl_2$, 6 sodium lactate, 10 HEPES, pH was adjusted to 7.3 with CsOH and osmolality was 290-300 mosmol/kg H_2O. The pipette solution contained (mM): 130 CsCl, 0.8 $MgCl_2$, 5 EGTA, 10 HEPES, 2 $CaCl_2$, 2 ATP-Mg^{2+}. Free Ca^{2+} concentration was set at $1.02x10^{-7}$ M, calculated using the program WinMAXC32 (Stanford University, Stanford, USA). The pH was adjusted to 7.2 with CsOH and osmolality was 280-290 mosmol/kg H_2O.

2.4. Voltage-dependent potassium currents

K^+ currents were measured in response to voltage steps (10 mV increments) between -80 mV and +80 mV for 200 ms, every 3 s, from a holding potential -40 mV. I-V curve of the steady state current was also fitted by a Boltzmann function (Eq. (1)). The time constant of activation (τ_{act}) of K^+ currents was obtained in response to a voltage step to +80 mV from a holding potencial -40 mV, adjusting a single exponential function describing the current curve using the same Eq. (2).

The external bath solution for measuring potassium currents contained (mM): 140 NaCl, 4.6 KCl, 1.6 $CaCl_2$, 1.13 $MgCl_2$, 10 D-glucose, 5 $NaHCO_3$, 10 HEPES, pH was adjusted to 7.4 with KOH and osmolality was 290-310 mosmol/kg H_2O. The pipette solution contained (mM): 150 KCl, 1 $MgCl_2$, 5 EGTA, 10 HEPES, 3 $CaCl_2$. Free Ca^{2+} concentration was set at $1x10^{-6}$ M. The pH was adjusted to 7.4 with KOH and osmolality was 290-310 mosmol/kg H_2O.

2.5. Statistical analysis

Results were expressed as mean ± standard error. The nonparametric test of Kruskal-Wallis with Dunn post-hoc was used to analyze different cells in the groups pre-treated for 2 h with heat stress or the Hsp90 inhibitor. Cells controls of themselves were submitted to the nonparametric Wilcoxon paired test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. T-type calcium currents

Fig. 1A shows the raw traces of Ca^{2+} currents in control Leydig cells. Long term exposure (2 h) of cells to HS or the Hsp90 inhibitor did not change calcium currents in comparison with control values (Fig. 1B and C). Short term exposure to the Hsp90 inhibitor also did not change the Ca^{2+} currents in either control or heat treated cells (Fig. 1D). Fig. 1E shows that exposure to the Hsp90 inhibitor (arrow) did not significantly change the peak T-type Ca^{2+} currents even after 700 s of treatment.

Table 2 shows the parameters of T-type Ca^{2+} currents in Leydig cells exposed or not to heat stress or pre-treated with the Hs90 inhibitor (GA) for 2 h.

Short term exposure to the Hsp90 inhibitor also did not change the Ca^{2+} currents parameters (Table 3).

3.2. Voltage-dependent potassium currents

Fig. 2A shows the raw traces of VKC K^+ currents in control Leydig cells. When Leydig cells were exposed to HS (Fig. 2B), VKC steady state currents at +80 mV were reduced by 45.3% (Cont: 40.6 ± 4.8[a], GA:

Table 2
Parameters of T-type Ca^{2+} currents in Leydig cells exposed or not to heat stress (HS) and/or pre-treated with geldanamycin (GA) for 2 h (R^2_{mean} = 0.99). G_{min} = minimum conductance, G_{max} = maximum conductance, V_0 = voltage at which half the channels are open, K = voltage dependence of the system, τ_{act} = time constants of activation, τ_{inact} = time constants of inactivation.

	Not exposed to heat stress		Exposed to heat stress		p
	Control	GA	Control	GA	
G_{min} (nS pF^{-1})	0.05 ± 0.003	0.05 ± 0.002	0.05 ± 0.002	0.06 ± 0.002	0.84
G_{max} (nS pF^{-1})	0.11 ± 0.006	0.11 ± 0.004	0.11 ± 0.004	0.11 ± 0.004	0.83
V_0 (mV)	-32.9 ± 0.5	-32.7 ± 0.4	-31.6 ± 0.4	-31.3 ± 0.3	0.60
K	5.0 ± 0.1	5.2 ± 0.1	5.0 ± 0.1	5.5 ± 0.1	0.10
τ_{act} (ms)	3.4 ± 0.2	3.2 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	0.61
τ_{inact} (ms)	17.5 ± 0.9	16.9 ± 1.0	19.5 ± 1.2	17.2 ± 1.7	0.49

37.9 ± 3.5[a], HS: 18.4 ± 2.5[b], HSGA: 20.9 ± 3.0[b] pA pF^{-1}, $p < 0.000$). However, VKC currents did not change due to pre-treatment of the cells with the Hsp90 inhibitor for 2 h (Fig. 2C and E) or their treatment shortly after the control record (Fig. 2F). Note that in these two situations all groups exposed to the high temperature showed a great inhibition of the potassium currents, regardless of the addition time of GA, demonstrated in 40 cells exposed to heat and 40 unheated cells (Fig. 2E and F).

It is noteworthy that Leydig cells treated with both HS and the Hsp90 inhibitor showed a similar response to the cells exposed only to high temperature (Fig. 2B and D), indicating that only HS changed the functioning of VKC channels.

Leydig cells treated with the Hsp90 inhibitor also did not change the potassium currents even after 700 s of treatment (Fig. 2G).

Table 4 shows the parameters of VKC K^+ currents in Leydig cells exposed or not to heat stress or pre-treated with the Hs90 inhibitor (GA) for 2 h.

The maximum conductance of potassium currents reduced 68.9% in Leydig cells after 2 h of exposure to high temperature. The parameter V_0 was 30% greater in cells exposed to HS, indicating a shift in the conductance versus voltage curve to more depolarized potentials in Leydig cells exposed to HS. The time constant of activation also increased 31.9% in cells exposed to heat. However, cells pre-treated with the Hsp90 inhibitor for 2 h or exposed to the inhibitor shortly after the control record showed no changes in the K^+ currents.

Short term exposure to the Hsp90 inhibitor also did not change the K^+ currents parameters in both normal cells or cells treated with HS (Table 5). These cells are controls of themselves, using a paired test with the same cell in a control record and recording shortly after addition of the Hsp90 inhibitor.

It is worth noting the alteration of the parameters between the groups exposed to HS and not exposed to HS, regardless of the treatment with the Hsp90 inhibitor.

3.3. Temperature variation during heat stress

The results obtained in the present study occurred due to the cellular response to environmental stress caused by the high temperature 2 h previously the analysis and not as a result of the high temperature itself. This assertion is supported by the fact that the electrophysiological experiments were performed only after returning the Leydig cells to room temperature for 2 h (120 min) after the HS. Note that around 60 min after HS the cells are already at room temperature, as shown in Fig. 3.

4. Discussion

Cryptorchidism and testicular heat exposure induces reversible oligospermia or azoospermia in animals and humans via increased germ cell apoptosis, as well as induce Leydig cell hyperplasia and reduction in testosterone biosynthesis (Li et al., 2015; Ayaz et al., 2018). Heat treatment also damage Leydig cells, induced a decrease in 3β-HSD expression, steroidogenic enzymes and progesterone, as well as an increase in GRP78/BiP and CHOP levels (Kim et al., 2016; Oka et al., 2017).

The inward and outward movement of ions through channels in the plasma membrane of cells works as signals in several physiological and pathological processes. Calcium and potassium currents are normally present in Leydig cells. Studies showed that T-type calcium channels and K_{Ca} potassium channels play an essential role in the steroidogensis of Leydig cells (Costa and Varanda, 2007; Matzkin et al., 2013). Furthermore, calcium channel blockers may impair normal spermatogenesis and steroidogenesis (Lee et al., 2011).

Although ion channels in Leydig cells are known to be involved in steroidogenesis, we did not find previous studies evaluating the function of K^+ or Ca^{2+} channels after exposure to adverse cellular conditions such as heat and cellular stress factors such as Hsp90.

All the ionic currents measured in the present study were performed in a Faraday cage and controlled environment to avoid electromagnetic noise, in addition, the signal was filtered to remove possible noises. These ionic currents are in accordance with the records shown in Costa et al. (2010) and de Deus et al. (2018).

Table 3
Parameters of T-type Ca^{2+} currents in Leydig cells exposed or not to heat stress (HS). Treatment with geldanamycin (GA) was performed shortly after the control record (R^2_{mean} = 0.99). These cells are controls of themselves. G_{min} = minimum conductance, G_{max} = maximum conductance, V_0 = voltage at which half the channels are open, K = voltage dependence of the system, τ_{act} = time constants of activation, τ_{inact} = time constants of inactivation.

	Not exposed to heat shock		p	Exposed to heat shock		p
	Control	GA		Control	GA	
G_{min} (nS pF^{-1})	0.05 ± 0.004	0.05 ± 0.004	0.72	0.04 ± 0.004	0.04 ± 0.002	0.13
G_{max} (nS pF^{-1})	0.10 ± 0.009	0.10 ± 0.009	0.67	0.09 ± 0.008	0.08 ± 0.01	0.13
V_0 (mV)	-31.8 ± 1.0	-33.1 ± 0.8	0.65	-28.8 ± 0.7	-29.1 ± 0.8	0.66
K	5.4 ± 0.2	4.9 ± 0.1	0.25	5.2 ± 0.2	4.9 ± 0.1	0.16
τ_{act} (ms)	3.3 ± 0.3	3.1 ± 0.2	0.12	4.8 ± 0.3	4.6 ± 0.2	0.97
τ_{inact} (ms)	15.2 ± 1.8	14.5 ± 1.8	0.30	20.6 ± 1.8	21.2 ± 2.2	0.30

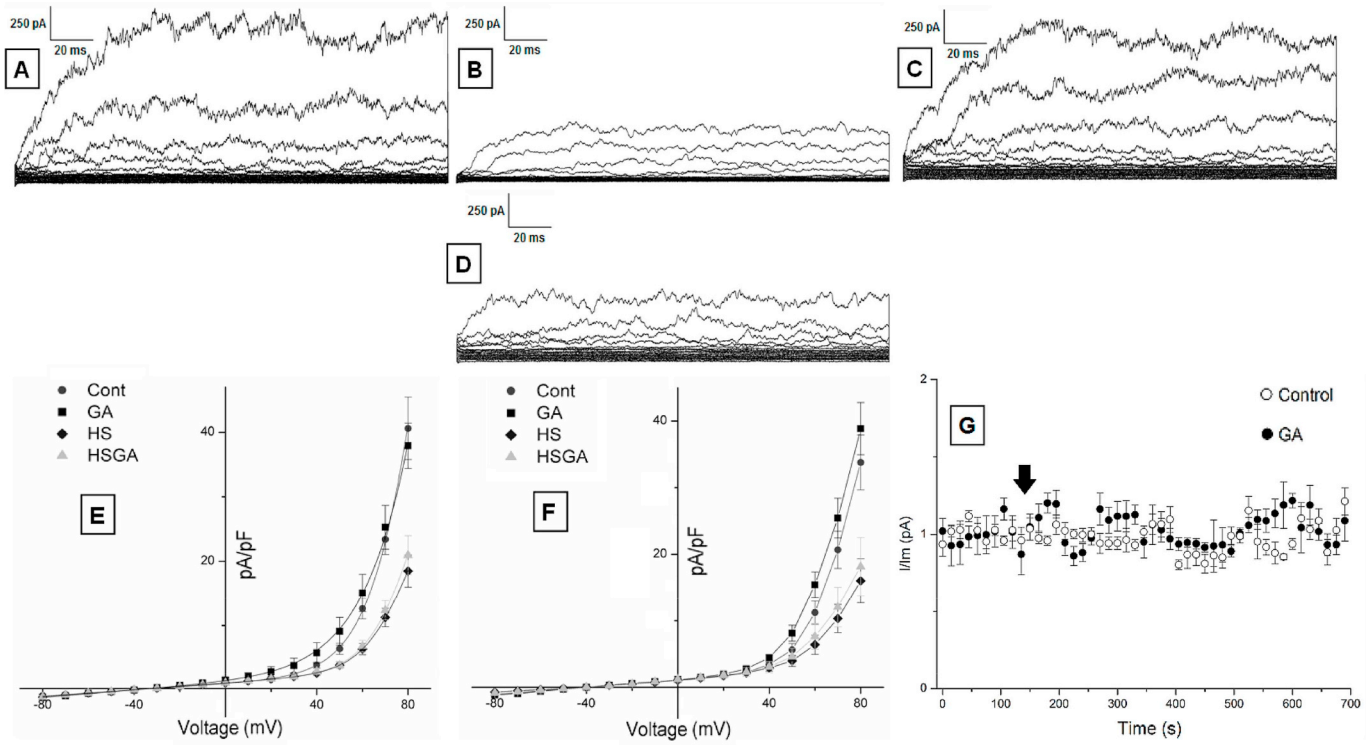

Fig. 2. Voltage-dependent potassium currents in mice Leydig cell obtained in response to voltage steps between -80 mV and +80 mV from a holding -40 mV. 2A: Raw traces of K^+ currents in control Leydig cells. 2B: K^+ currents 2 h after heat stress (HS). 2C: K^+ currents in cells not exposed to HS but pre-treated for 2 h with geldanamycin (GA, Hsp90 inhibitor). 2D: K^+ channel currents 2 h after exposure to both HS and GA (HSGA). 2E: I-V relationships of control cells (Cont) or cells exposed to HS and/or GA for 2 h (n_{Cont} = 10, n_{GA} = 10, n_{HS} = 10, n_{HSGA} = 10 cells). 2F: I-V relationships of cells treated with GA shortly after the control records (n_{Cont} = 10, n_{GA} = 10, n_{HS} = 10, n_{HSGA} = 10 cells). 2G: Steady state currents of K^+ channels elicited by voltage pulses to +80 mV, every 15 s, from a holding -40 mV. Arrow indicates the time of addition of GA to the bathing solution (n = 8). Values were normalized by the average of the points obtained in control conditions.

4.1. Effect of heat stress on T-type calcium and VKC potassium currents in leydig cells

Cellular stress responses are universal mechanisms of extraordinary physiological and pathological significance and represent defense reactions of cells to environmental damage in their macromolecules. Although many factors are involved in the cellular responses to stress, several aspects of this response are not stressor specific because cells monitor stress based on damage to macromolecules independent of what kind of stress caused the cellular damage (Herr and Debatin, 2001; Kültz, 2005). The present study demonstrated that voltage-dependent potassium currents can be inhibited by HS, suggesting a relationship of these currents with the stress response of Leydig cells to heat.

Although studies reported that Leydig cells and testosterone production can be altered by adverse conditions, such as heat (Li et al., 2015; Kim et al., 2016), Leydig cells are considered more thermo-resistant than germ cells (Setchell, 2006). Acute heat stress is known to obstruct testicular steroidogenesis; in contrast, chronic heat stress such as cryptorchidism or varicocele generally does not affect testicular steroidogenesis, suggesting that Leydig cells adapt to heat stress and retain their steroid synthesis ability, but the mechanisms of the stress response in this adaptation are unclear (Oka et al., 2017). Our results suggest a possible role for the voltage-dependent potassium currents in the process of response to heat by Leydig cells.

After thermal stress in the testis, germ cells die via apoptosis, which may lead to infertility. Meanwhile, Leydig cells in the testis are more thermo resistant and generally do not die via apoptosis (Setchell, 2006; Tenorio et al., 2014). According to Armstrong and Robertson (2006), K^+ regulatory mechanisms may be an important line of defense against heat stress. K^+ participates in the beginning, middle and end of apoptosis; K^+ efflux through the plasma membrane causes a reduction in cell volume during apoptosis, which is a necessary initial step for triggering the apoptotic process (Burg et al., 2006). Considering that apoptosis can be affected by K^+ channels modulation (Yu et al., 1997), we suggest a

Table 4
Parameters of voltage-dependent potassium currents in Leydig cells exposed or not to heat stress (HS) and/or pre-treated with geldanamycin (GA) for 2 h (R^2_{mean} = 0.99). G_{min} = minimum conductance, G_{max} = maximum conductance, V_0 = voltage at which half the channels are open, K = voltage dependence of the system, τ_{act} = time constants of activation.

	Not exposed to heat shock		Exposed to heat shock		p
	Control	GA	Control	GA	
G_{min} (nS pF^{-1})	0.02 ± 0.007	0.03 ± 0.008	0.02 ± 0.011	0.02 ± 0.005	0.95
G_{max} (nS pF^{-1})	1.0 ± 0.3[a]	0.7 ± 0.2[a]	0.2 ± 0.06[b]	0.2 ± 0.07[b]	0.00[a]
V_0 (mV)	81.5 ± 4.3[a]	85.2 ± 16.5[a]	120.5 ± 5.9[b]	117.7 ± 3.0[b]	0.00[a]
K	11.6 ± 1.2	12.2 ± 2.5	9.4 ± 1.2	9.6 ± 0.9	0.51
τ_{act} (ms)	14.5 ± 1.3[a]	14.9 ± 1.2[a]	19.1 ± 1.5[b]	20.6 ± 3.6[b]	0.02[a]

[a] Statistically significant.

Table 5
Parameters of voltage-dependent potassium currents in Leydig cells exposed or not heat stress (HS). Treatment with geldanamycin (GA) was performed shortly after the control record (R^2mean = 0.99). These cells are controls of themselves. G_{min} = minimum conductance, G_{max} = maximum conductance, V_0 = voltage at which half the channels are open, K = voltage dependence of the system, τ_{act} = time constants of activation.

	Not exposed to heat shock		p	Exposed to heat shock		p
	Control	GA		Control	GA	
G_{min} (nS pF^{-1})	0.02 ± 0.002	0.02 ± 0.002	0.92	0.02 ± 0.002	0.02 ± 0.005	0.98
G_{max} (nS pF^{-1})	0.7 ± 0.07	0.7 ± 0.1	0.92	0.2 ± 0.05	0.2 ± 0.08	0.35
V_0 (mV)	73.3 ± 2.7	74.4 ± 3.4	0.92	77.2 ± 2.8	83.9 ± 7.7	0.65
K	9.6 ± 0.9	11.8 ± 0.8	0.16	13.1 ± 3.0	11.0 ± 1.5	0.97
τ_{act} (ms)	15.0 ± 1.0	13.8 ± 1.1	0.23	19.5 ± 2.2	18.4 ± 1.4	0.96

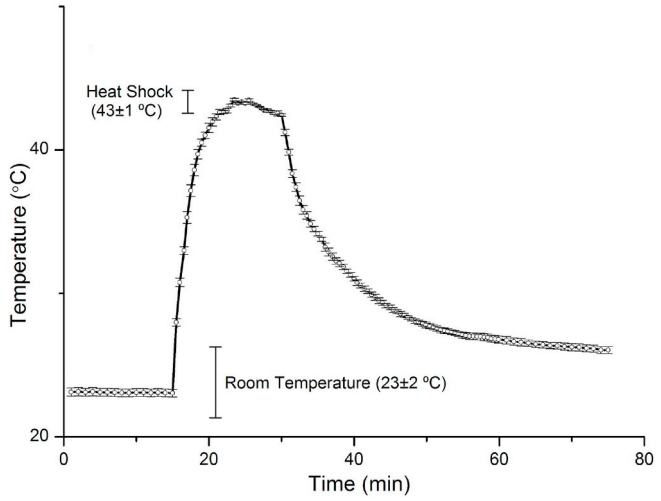

Fig. 3. Time course of temperature variation of the solution containing the Leydig cells during heat stress treatment at 43 ± 1 °C for 15 min (n = 6). Note that the electrophysiological analyses were performed 2 h after heat stress (120 min), when the temperature was already right back to room temperature.

possible role for the reduction of the K^+ currents to help prevent apoptosis in Leydig cells exposed to HS.

Studies showed an increase in K^+ currents with increasing temperature in several cell types, as well as a faster activation and inactivation kinetics at higher temperatures (Pahapill and Schlichter, 1990; Zhou et al., 1998). On the other hand, in the present study we observed an inhibition of K^+ currents due to HS after 2 h at room temperature, indicating that others factors than temperature inhibited the K^+ currents.

We did not find previous studies reporting the inhibition of voltage-dependent potassium currents in Leydig cells exposed to heat stress. Furthermore, only a few studies have evaluated the effect of HS on potassium currents in other cell types. Results observed in the present study corroborate those of Ramirez et al. (1999), Wu et al. (2001), Wu et al. (2002) and Robertson (2004), who showed reduced neuronal K^+ currents in nerve cells exposed to heat stress. Duncker, 2000, Hoag et al. (1997) and Joyeux et al. (1998) reported that the activation of ATP-sensitive potassium channels protect the function of heart cells 24 h after the exposure to heat stress. Saad and Hahn (1992) showed a correlation between heat resistance and expression of K^+ channels. The calcium-activated chloride channel also acts as a heat sensor in nociceptive neurons (Cho et al., 2012). According to Armstrong and Robertson (2006), little attention has been given to possible mechanisms coordinating tissue-specific responses to hyperthermic stress.

4.2. Effect of Hsp90 inhibition on T-type calcium and VKC potassium currents in leydig cells

Heat shock proteins (Hsp) are factors of cellular response to stress very conserved among species, from bacteria to mammals. Hsp's are expressed at low levels in physiological conditions and high levels during cellular stress, helping cells to survive the environmental challenges (Kalmar and Greensmith, 2009). Despite the importance of Hsp90 in cells, the present study did not observe changes in Ca^{2+} or K^+ currents after Hsp90 inhibition in Leydig cells.

On the other hand, some studies reported that Hsp's can interact with ion channels of different cell types, such as: K^+_{ATP} channels in pancreatic β cells (Yan et al., 2010), K^+ channels hERG in HEK-293 cells (Walker et al., 2010) and K^+ channels hERG in cells subjected to hypoxia (Nanduri et al., 2009).

In the present study the geldanamycin analogue 17-DMAP-GA was used as the Hsp90 inhibitor due to the following pharmacological characteristics: 1) Satisfactory inhibition capacity of Hsp90; 2) Water soluble, allowing its use without addition of DMSO; 3) Less toxic, preventing indirect cellular effects (Tian et al., 2004). The difference between our results and those mentioned above may be due to: 1) Intrinsic differences in cell types, because Leydig cells have resistance to environmental stress, maintaining their functional capacity (Setchell, 2006); 2) Use of different geldanamycin analogues; 3) Duration of pretreatment with Hsp90 inhibitor, because we used fresh isolated Leydig cells which did not allow a prolonged exposure to the inhibitor but maintains the physiological characteristics of the cell.

In conclusion, exposure to HS reduces the VKC potassium currents, leading to a slower activation and reducing their voltage dependence. T-type calcium currents were not changed by high temperature exposure. Administration of the Hsp90 inhibitor (17-DMAP-geldanamycin) did not change T-type calcium or VKC currents. HS inhibits K^+ currents, which may be a signaling or protective mechanism involved in the response to stress in Leydig cells. Hsp90 does not seem to be involved in this process.

Declaration of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.05.022.

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