ORIGINAL ARTICLE

Hormonal Control of Cardiac Action Potential Phase 1 Currents in the Brugada Syndrome

Control hormonal de las corrientes de la fase 1 del potencial de acción cardíaco en el síndrome de Brugada

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ABSTRACT

Introduction: The Brugada syndrome is an inherited channelopathy with autosomal dominant genotype transmission pattern presenting marked gender bias in phenotype expression, with a male to female ratio of 9:1. A cellular model of the disease suggests a heterogeneous distribution in the phase 1 amplitude of the ventricular action potential as the origin for the development of the arrhythmogenic substrate.
Objective: The aim of this study was to investigate the role of androgens on the cardiac action potential phase 1 regulation and its electrophysiological consequences in an experimental murine model of Brugada syndrome.
Methods: Androgen control of gene expression was studied in HL-1 cells and rat hearts using real time polymerase chain reaction (PCR). For the electrophysiological studies, an experimental model of the Brugada syndrome was reproduced in a Langendorff system using Tyrode solution supplemented with pinacidil and terfenadine.
Results: Treatment of HL-1 cells with dihydrotestosterone increased the expression of the Kv4.3 potassium channel and the sodium/calcium exchanger (NCX). This effect was assessed in rats treated with testosterone and finasteride. The expression of both genes decreased with finasteride, whereas testosterone increased NCX messenger ribonucleic acid (mRNA) level. Testosterone produced action potential shortening at 90% repolarization (APD90) and decreased time to peak (TTP), which in Brugada syndrome models correlate with increased arrhythmogenesis. In our model, this phenomenon was observed both as an increase of sporadic and sustained ectopic ventricular action potentials. The frequency of ectopic action potentials induced with terfenadine and pinacidil in the control group was reduced by an order of magnitude with finasteride treatment.
Conclusions: Androgens control the expression of key components of the cardiac action potential resulting in increased arrhythmogenesis. Finasteride treatment reverses these effects.

Key words: Brugada Syndrome - Cardiac arrhythmias - Androgens - Finasteride

RESUMEN

Introducción: El síndrome de Brugada es una canalopatía hereditaria con un patrón de transmisión autosómico dominante que presenta un marcado sesgo de género en la expresión del fenotipo, con una proporción hombre:mujer de 9:1. Un modelo celular de la enfermedad propone una distribución heterogénea de la amplitud de la fase 1 del potencial de acción ventricular como la base para el desarrollo del sustrato arritmogénico.
Objetivo: Investigar el papel de los andrógenos en la regulación de la fase 1 del potencial de acción cardíaco en ratas y sus consecuencias electrofisiológicas en un modelo experimental murino del síndrome de Brugada.
Material y métodos: Se estudió el control de la expresión génica por andrógenos en células HL-1 y en corazones de rata por reacción en cadena de la polimerasa (PCR) en tiempo real. Para los estudios de electrofisiología se reprodujo un modelo experimental del síndrome de Brugada en un sistema de Langendorff utilizando solución de Tyrode suplementada con pinacidil y terfenadina.
Resultados: El tratamiento de células HL-1 con dihidrotestosterona produjo un aumento en la expresión del canal del potasio Kv4.3 y del intercambiador de sodio/calcio (NCX). Se evaluó este efecto en ratas tratadas con testosterona y finasterida. La expresión de ambos genes se redujo con la finasterida, mientras que la testosterona aumentó el nivel de ácido ribonucleico mensajero (ARNm) del NCX. La testosterona produjo un acortamiento de la duración del potencial de acción a 90% de la repolarización (APD90) y del tiempo al pico (TTP), lo cual en modelos del síndrome de Brugada se correlaciona con un aumento de la arritmogenicidad. En nuestro modelo, este fenómeno se observó como un incremento en los potenciales de acción ventriculares ectópicos, esporádicos y sostenidos. La frecuencia de aparición de potenciales de acción ectópicos inducida con terfenadina y pinacidil en el grupo control se redujo en un orden de magnitud con el tratamiento con finasterida.
Conclusiones: Los andrógenos controlan la expresión de componentes clave del potencial de acción cardíaco, con el resultado de un aumento de la arritmogenicidad. El tratamiento con finasterida revierte estos efectos.

Palabras clave: Síndrome de Brugada – Arritmias cardíacas - Andrógenos - Finasterida.

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Abbreviations

APD90	Action potential duration at 90% repolarization	**NCX**	Sodium/calcium exchanger
RNA	Ribonucleic acid	**EVAP**	Ectopic ventricular action potentials
mRNA	Messenger ribonucleic acid	**PCR**	Polymerase chain reaction
RSEVAP	Runs of sustained extrasystolic ventricular action potentials	**AR**	Androgen receptor
DHT	Dihydrotestosterone	**TTP**	Time to peak

INTRODUCTION

The Brugada syndrome is characterized by ST-segment elevation of electrocardiographic right precordial leads and is often associated with right bundle branch block. Clinically, it presents increased risk of cardiac arrhythmias and sudden death in the absence of structural heart disease. (1)

The Brugada syndrome is an inherited channelopathy transmitted with a dominant autosomal pattern. Mutations in the SCN5A gene that encodes the alpha subunit of the sodium channel have been found in 20% of affected individuals. (2) The disease has variable expression, and is 8 to 10 times more prevalent in men than women. (3)

Aside from the genetic diversity, an underlying common mechanism explains the origin of ventricular arrhythmias: a non-homogeneous loss of the epicardial phase-2 dome causing transmural and epicardial dispersion of repolarization. The arrhythmogenic substrate generates as consequence of dome propagation from the sites where it persists towards those where it has been lost. (4, 5)

The predominance of the Brugada syndrome in the male population has a hormonal basis. The reversion of the Brugada syndrome phenotype in two patients with prostate cancer after surgical castration observed by Matsuo et al. (2003) points out the role of androgens in the development of the disease. (6) In accordance with this finding, male patients with Brugada syndrome have been reported to have higher testosterone levels than age-matched normal controls. (7)

The Ito current results from the activity of several channels and regulators. Among these, the Kv4.3 potassium channel plays a key role. (10) Its expression is greater in men than in women, in the right than in the left ventricle and in the epicardium than in the endocardium (8, 9, 11). In humans, Kv4.3 activity is almost the sole component of Ito, whereas in the rat Kv4.2 and Kv4.3 participate equally from Ito, with a lower contribution of Kv1.4. In this case, the transmural gradient of Ito does not depend on the gradient of Kv4.3 activity, but on the expression of Kv4.2. (12)

Another component of the phase 1 depolarization current is the Na+/C2+ exchanger (NCX) working in reverse mode. During phase 1 and the beginning of phase 2, the NCX equilibrium potential is above that of the membrane potential. This makes it work in reverse mode extruding 3 Na+ for 1 Ca2+, producing a hyperpolarizing current that contributes to the phase 1 amplitude. (13)

Based on these findings, we postulate that androgen regulation of action potential ionic currents could be a key factor in the expression of Brugada syndrome phenotype. To test this hypothesis, we assessed: 1) the differential androgen regulation on phase 1 components and the Cacna1c Ca2+ channel (main component of ICaL) and 2) the effects of androgen activity on arrhythmogenesis in an experimental model of Brugada syndrome.

METHODS

Cell cultures

HL-1 cells were cultured at 37 °C and 5% CO2 in Claycomb media (Sigma Aldrich, USA) supplemented with 100 mM norepinephrine, 4 mM L-glutamine and 10% fetal bovine serum, in the presence or absence of 1 mM dihydrotestosterone (DHT, Steraloids, USA).

Animals

Housing: fifteen, three-month old, male Wistar rats were used. The animals were kept in a temperature and humidity controlled environment with a 12/12 hour light/darkness cycle and free access to food and water.

Euthanasia: the animals were anesthetized with vaporized 5% Enflurane (Inheltran®, Abbott, Italy) until absence of reflexes and then sacrificed by cervical dislocation.

This research was approved by the Ethics Evaluation Committee of Universidad Nacional de General San Martín in accordance with the Revised guide for the care and use of laboratory animals (NIH GUIDE, Volume 25, Number 28, August 16, 1996).

Drug administration

Rats were randomly assigned to three groups (n = 5 in each group): a control group that did not receive any treatment; the finasteride group that received an intramuscular injection of 2 mg finasteride (Sigma Aldrich, USA), and the testosterone group that received an intramuscular injection of 5 mg testosterone (Sigma Aldrich, USA). Injections were delivered once daily for a week, using supra-maximum doses to saturate the receptors.

RNA isolation and real time polymerase chain reaction

Samples were treated with Trizol (Invitrogen) to extract total RNA. Reverse transcription was performed using random primers (Biodynamics) and RevertAid M - MuLV (Fermentas) according to the manufacturer´s instructions.

The genetic expression by real time polymerase chain reaction (PCR) was analyzed using SYBR Green Master Rox (Roche) reaction mixture and specific primers for each gene (Table 1).

Polymerase chain reactions were performed using StepOne™ equipment (Applied Biosystems™) with an initial 2 min denaturation cycle at 95º C, followed by thermal cycling: 20 seconds at 95º C, 1 minute at 60º C and 20 seconds at 72º C.

Messenger RNA (mRNA) abundance was normalized to the expression of the glyceraldehyde 3-phosphate

dehydrogenase (GAPDH) constitutive gene according to the method described by Pfaffl. (14)

Electrophysiologic measurements in the isolated, perfused right ventricle

The isolated right ventricle was perfused through the aortic artery with oxygenated Tyrode solution at 37º C. Epicardial action potentials were recorded using a floating microelectrode filled with 3 M KCl (FD223 electrometer, WPI, Sarasota, Florida). All drugs were dissolved in Tyrode solution and were perfused through the aorta.

The preparation was stimulated at 5 Hz with a rectangular waveform pulse of 30 ms duration (Pulsemaster A300, WPI, Sarasota, Florida). The arrhythmia protocol was: 10 seconds at 10 Hz, 10 seconds at 20 Hz and 1 minute at 0.5 Hz (when the measurements were acquired).

The protocol described by Di Diego et al. (8) to simulate the Brugada syndrome was adapted for its use in male Wistar rats. Following stabilization, the ventricles were perfused with Tyrode solution, 5 μM pinacidil and 5 μM terfenadine. Preparations not exhibiting ectopic ventricular action potentials (EVAP) during a 2-hour stimulation period and arrhythmia protocols were considered negative. In the context of this model we defined runs of sustained extrasystolic ventricular action potentials (RSEVAP) as the sequence of 20 or more spontaneous action potentials, and they were considered separately from EVAP which never exceeded 10 consecutive action potentials.

Statistical analysis

Results are expressed as mean ± standard error (SE), after goodness of fit test to assess normal distribution. Data were analyzed using ANOVA followed by Student-Newman-Keuls or Bonferroni correction post-hoc analyses, as applicable. A two-tailed p value < 0.05 was considered as statistically significant. Statistical analyses were performed using SPSS Statistics 17.0 software.

RESULTS

Androgen control of Kv4.3 and NCX

Dihydrotestosterone regulates phase 1 channels in cardiac cells

The HL-1 cellular line derived from murine cardiomyocytes was used to evaluate the role of DHT in the regulation of the expression level of the genes of interest by real time PCR. Culture media supplemented with DHT significantly increased KCND3 and NCX1 mRNA levels. The treatment did not modify CACNA1c, KCNA4 and KCND2 expression (Table 2).

KCND3 and NCX1 expression in the rat heart: effect of testosterone and finasteride treatment

Based upon results obtained with the cell line, an experiment in male Wistar rats was designed to assess the effect of testosterone and DHT on KCND3 and NCX1 expression. Accordingly, a group treated with finasteride, inhibitor of 5-α-reductase, responsible for DHT synthesis from testosterone, was added to the control and exogenous testosterone groups. Blood testosterone levels were kept within the normal range in the control and finasteride groups (1.36 ± 0.21 and 1.50 ± 0.17 ng/ml, respectively). In the group treated with testosterone at saturation dose, concentrations > 15 ng/ml were observed in all cases.

At the transcriptional level, finasteride significantly reduced the expression of both genes. Conversely, the group treated with testosterone increased the expression of NCX1 but not of KCND3 (Table 3).

Effect of androgenic hormones in a Brugada syndrome model

The protocol described by Di Diego et al. (8) to simulate the Brugada syndrome was adapted to generate a moderate arrhythmogenic response in control rats (untreated). Terfenadine (sodium and calcium channel blocker) combined with pinacidil (KATP channel activator) was used. Within the framework of this model, action potentials generated in response to an arrhythmia protocol were recorded to assess the electrophysiologic effects of testosterone and finasteride treatment.

Variation in general action potential parameters

The antagonistic action of testosterone and finasteride was reflected in action potential time to peak (TTP) and APD90 (Figure 1A and B). However, although the same tendency was observed in both parameters, finasteride did not significantly increase APD90 with respect to control.

In the case of the initial phase of repolarization (APD30), only treatment with finasteride produced significant changes (Figure 1C). No changes were seen in the resting potential (RP, Figure 1D).

Arrhythmia induction in the murine model of Brugada syndrome

Figure 2 shows representative right ventricular action potentials in the different groups following drug administration and the arrhythmia protocol.

The response of the arrhythmia protocol to stimulation varied among groups (Table 4). The reference result in the control group was that 4 out of 5 rats

Table 1. Specific gene primers

Gene	Forward primer (direct) 5`→3`	Reverse primer (inverse) 5`→3`
GAPDH	TGCATCCTGCACCACCAACT	CTTGGCAGCACCAGTGGATG
KCND3 (Kv4.3)	CCCTCACCATGGCCATCATC	AATGACCAGGACGCCGCTTA
NCX1 (NCX)	GGCTGGGCCTGCTTCATTGT	TTTCTGGCCTCCGCCGATAC
KCNA4 (Kv1.4)	CCACCTGCCAAACCTGAGCGATTT	GGGTGGACTCCCAGACCTTCCCTCT
CACNA1c (Cav1.2)	GGAGAGTCCAGCGAGAAACTCAA	CGGCGTTCTCCATCTCCTCTATT
KCND2 (Kv4.2)	ACCAGCACTTGCTGCTCACG	AGGGCTCCCATGCTCTCAGA

Table 2. Dihydrotestosterone-induced changes in the HL-1 cell line genetic expression

	Relative expression (mRNA)	
	Control	DHT
KCND3	1± 0.18	2.08 ± 0.17**
NCX1	1± 0.14	1.84 ± 0.26*
CACNA1c	1± 0.32	0.59 ± 0.17
KCNA4	1± 0.31	0.63 ± 0.09
KCND2	1± 0.28	0.77 ± 0.11

Messenger ribonucleic acid (mRNA) quantification by real time polymerase chain reaction in HL-1 cells. Dyhydrotestosterone (DHT) treatment increased KCND3 and NCX1 expression, but not KCNA4, KCND2 and CACNA1c expression. Data are expressed as mean ± standard error of the mean (* = $p < 0.05$ vs. control; ** = $p < 0.01$ vs. control; n = 6).

Table 3. Androgen effect on genetic expression in the rat right ventricle

	Relative expression (mRNA)		
	Control (n=5)	Testosterone (n=5)	Finasteride (n=5)
KCND3	1.03 ± 0.14	1.06 ± 0.09	0.68 ± 0.04*
NCX1	1.06 ± 0.20	1.52 ± 0.18*	0.69 ± 0.13*

Messenger ribonucleic acid (mRNA) quantification by real time polymerase chain reaction in the rat right ventricle. Finasteride reduced the expression of both genes, whereas treatment with testosterone significantly increased NCX1 expression. Data are expressed as mean ± standard error of the mean (* = $p < 0.05$ vs. control; n = 5).

developed EVAP in response to the arrhythmia protocol. No RSEVAP were observed in this group. Testosterone treatment produced a marked increase of arrhythmogenesis, as all the rats in this group showed EVAP and 2 out of 5 animals presented RSEVAP. Finasteride treatment significantly reduced ectopic action potentials, as none of the hearts developed RSEVAP and only one presented EVAP. (see Table 4).

DISCUSSION

The Brugada syndrome has been postulated to be generated by ion current changes during action potential initiation, especially phase 0 and phase 1 ion currents. (8) In the present study, a rat model was used to describe androgen effect on the regulation of genes that could affect disease development. We observed that DHT specifically increased Kv4.3 and NCX1 expression in HL-1 cells and that finasteride produced a marked mRNA reduction from these genes in the rat heart. On the other hand, testosterone effect was restricted to the NCX1 gene. Moreover, Kv4.3 has been found to be also regulated by estrogens (15, 16), which together with our results suggest that the resulting level of expression would be subject to hormonal balance and not directly to the effect of the androgenic hormone.

At the electrophysiologic level, testosterone treatment produced APD90 and TTP shortening, which in Brugada syndrome models correlate with increased arrhythmogenesis. (17) As finasteride induced the reverse effect (APD90: 31.91 ± 2.72 vs. 52.89 ± 2.80 and TTP: 3.73 ± 0.21 vs. 7.15 ± 0.50 testosterone vs.

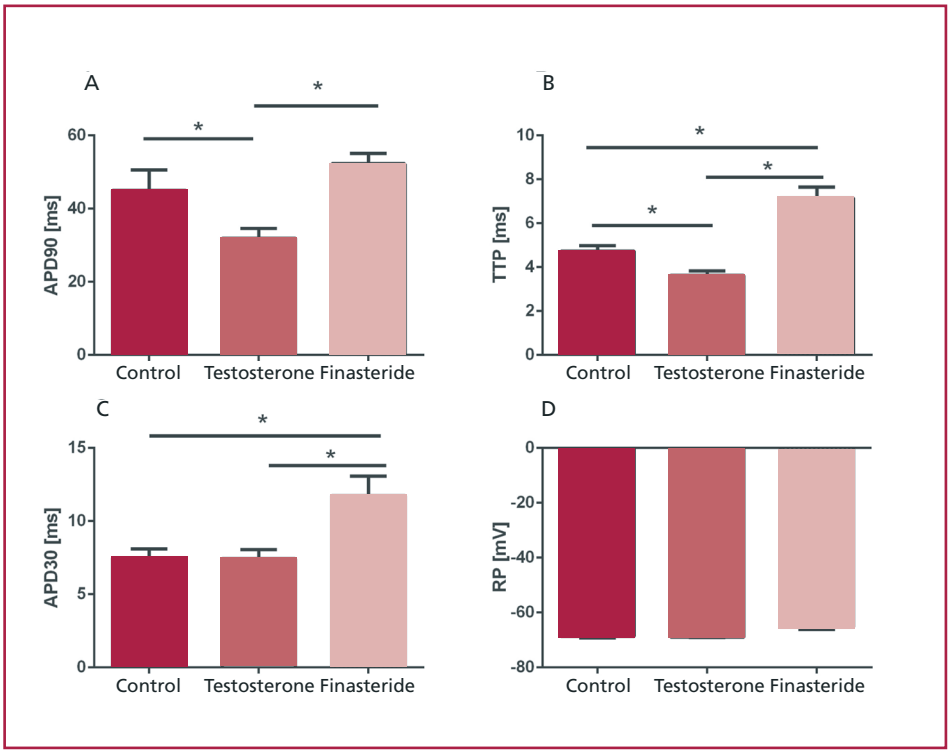

Fig. 1. Changes in action potential parameters in the rat right ventricle. APD90: action potential duration at 90% repolarization; APD30: action potential duration at 30% repolarization; TTP: time to peak; RP: resting membrane potential. * = $p < 0.05$; n=5).

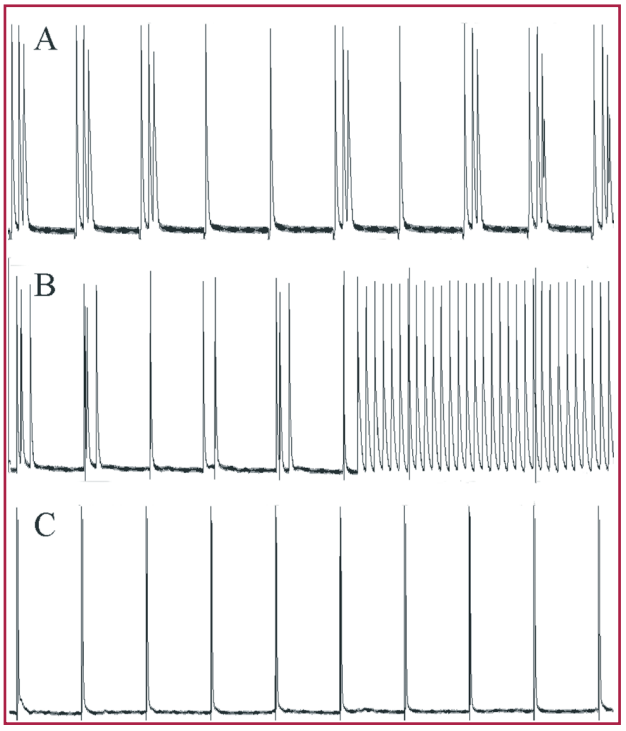

Fig. 2. Action potential recordings following terfenadine and pinacidil treatment and the arrythmia protocol. Panels **A, B** and **C** show representative action potentials following application of the arrhythmia protocol (10 Hz for 10 seconds, 20 Hz for 10 seconds and 0.5 Hz for 1 minute) in the isolated right ventricle, perfused with Tyrode solution supplemented with terfenadine and pinacidil, of control rats (**A**), rats treated with testosterone (**B**) and rats treated with finasteride (**C**).

Table 4. Arrhythmia induction in the Brugada syndrome model.

	Relative expression (mRNA)		
	Control	Testosterone	Finasteride
EVAP	4/5	5/5	1/5*#
RSEVAP	0/5	2/5	0/5
Positive protocols (%)	44.4 ± 5.5	69.7 ± 6.1*	3.7 ± 1.7*#

Ectopic ventricular action potentials (EVAP) and runs of sustained extrasystolic ventricular action potentials (RSEVAP) were quantified in the right ventricle of the rat. Results are expressed as number of animals presenting at least one EVAP or RSEVAP/N. The table also shows the percentage of positive protocols observed during a 2-hour stimulation period and arrhythmia protocols (* = $p < 0.05$ vs. control; # = $p < 0.05$ testosterone vs. finasteride).

finasteride $p< 0.01$) it may be concluded that in this model androgenic activity correlates directly with an increase of parameters evaluating arrhythmogenesis. This was also reflected in the response to stimulation by the arrhythmia protocol, where finasteride was able to reduce by one order of magnitude the frequency of positive protocols with respect to control.

One of the major limitations in the study of Brugada syndrome is lack of a model in small laboratory animals, as both the action potential and channel activity vary among species. (18, 19) The rat action potential does not have the human phase 2 dome and the Ito current acts mainly on the early repolarization phase. In this sense, the increase in APD30 induced by finasteride may be attributed to decreased Kv4.3 and NCX1 activity.

The present work postulates a relevant role for the NCX in the development of Brugada syndrome. In the rat, NCX acts in the reverse mode during phase 1 generating a hyperpolarizing current until the membrane potential becomes lower than its equilibrium potential. In humans, the NCX continues working in the reverse mode during phase 2. (20) Therefore, it is possible that greater NCX activity increases phase 1 intensity, contributing to loss of the phase 2 Ca2+ plateau. Although a direct extrapolation of these results to the human action potential is not possible, it could be assumed that a reduction of Ito could avoid dome loss.

In the context of this evidence, the present results on the androgenic regulation of Kv4.3 and NCX indicate a mechanism by which androgen levels facilitate the development of the Brugada syndrome. The transcriptional activity of androgen receptors (AR) is the result of the interaction of their agonists, testosterone and DHT. Both agonists evidence additive effects on AR. Testosterone is more abundant than DHT, but DHT has higher affinity for AR. (21) Thus, finasteride decreases indirectly the transcriptional activity of AR, which is reflected in the reduced expression of both Kv4.3 and NCX in the rat right ventricle.

Numerous efforts have been done during the last years to find a treatment for the Brugada syndrome. However, whereas antiarrhythmic agents such as amiodarone and β-blockers have been ineffective, specific Ito inhibitors with higher healing power (among them 4-aminopyridine and quinidine) produce secondary effects that prevent their therapeutic use. We consider that our results, within the constraints imposed by the rat model, deserve future studies analyzing the effect of finasteride in Brugada syndrome patients. It should be pointed out that finasteride is currently an over the counter drug for which no adverse effects have been described.

Conflicts of interest
None declared.

REFERENCES

1. Benito B, Brugada R, Brugada J, Brugada P. Brugada síndrome" Prog Cardiovasc Dis 2008;51:1-22. http://doi.org/ddg7kw
2. Nielsen MW, Holst AG, Olesen SP, Olesen MS. The genetic component of Brugada Syndrome. Front Physiol 2013;15:179.
3. Berne P, Brugada J. Brugada síndrome Circ J 2012;76:1563-71. http://doi.org/ddg7kw
4. Antzelevitch C. Molecular biology and cellular mechanisms of Brugada and long QT syndromes in infants and young children. J Electrocardiol 2001;34 Suppl:177-81. http://doi.org/b6sg45
5. Antzelevitch C, Yan GX, Shimizu W. Transmural dispersion of repolarization and arrhythmogenicity: the Brugada syndrome versus the long QT syndrome. J Electrocardiol 1999;32 Suppl:158-65. http://doi.org/cjtn39
6. Matsuo K, Akahoshi M, Seto S, Yano K. Disappearance of the

Brugada-type electrocardiogram after surgical castration: a role for testosterone and an explanation for the male preponderance. Pacing Clin Electrophysiol 2003;26(7 Pt 1):1551-3. http://doi.org/dw79m3

7. Shimizu W, Matsuo K, Kokubo Y, Satomi K, Kurita T, Noda T, et al. Sex hormone and gender difference--role of testosterone on male predominance in Brugada syndrome. J Cardiovasc Electrophysiol 2007;18:415-21. http://doi.org/cbsr26

8. Di Diego JM, Cordeiro JM, Goodrow RJ, Fish JM, Zygmunt AC, Pérez GJ, et al. Ionic and cellular basis for the predominance of the Brugada syndrome phenotype in males Circulation 2002;106:2004-11. http://doi.org/bhfhnm

9. Di Diego JM, Sun ZQ, Antzelevitch C. I(to) and action potential notch are smaller in left vs. right canine ventricular epicardium. Am J Physiol 1996; 271:H548-61

10. Kong W, Po S, Yamagishi T, Ashen MD, Stetten G, Tomaselli GF. Isolation and characterization of the human gene encoding Ito: further diversity by alternative mRNA splicing. Am J Physiol 1998;275:H1963-70

11. Zicha S, Xiao L, Stafford S, Cha TJ, Han W, Varro A, Nattel S. Transmural expression of transient outward potassium current subunits in normal and failing canine and human hearts. J Physiol. 2004:15;561(Pt 3):735-48.

12. Teutsch C, Kondo RP, Dederko DA, Chrast J, Chien KR, Giles WR. Spatial distributions of Kv4 channels and KChip2 isoforms in the murine heart based on laser capture microdissection. Cardiovasc Res 2007;73:739-49. http://doi.org/bf7qrd

13. Carmeliet E. Intracellular Ca(2+) concentration and rate adaptation of the cardiac action potential. Cell Calcium 2004;35:557-73. http://doi.org/bvdscr

14. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:2002-7. http://doi.org/b7shzz

15. Saito T, Ciobotaru A, Bopassa JC, Toro L, Stefani E, Eghbali M. Estrogen contributes to gender differences in mouse ventricular repolarization. Circ Res 2009;14;105:343-52. http://doi.org/dq299z

16. Song M, Helguera G, Eghbali M, Zhu N, Zarei MM, Olcese R, et al. Remodeling of Kv4.3 potassium channel gene expression under the control of sex hormones. J Biol Chem 2001; 24;276:31883-90. http://doi.org/d4js7n

17. Sabir IN, Li LM, Jones VJ, Goddard CA, Grace AA, Huang CL. Criteria for arrhythmogenicity in genetically-modified Langendorff-perfused murine hearts modelling the congenital long QT syndrome type 3 and the Brugada syndrome. Pflugers Arch 2008;455:637-51. http://doi.org/c7z63x

18. Patel SP, Campbell DL. Transient outward potassium current, 'Ito', phenotypes in the mammalian left ventricle: underlying molecular, cellular and biophysical mechanisms. J Physiol 2005;569:7-39. http://doi.org/cgjhtw

19. Akar FG, Wu RC, Deschenes I, Armoundas AA, Piacentino V 3rd, Houser SR, et al. Phenotypic differences in transient outward K+ current of human and canine ventricular myocytes: insights into molecular composition of ventricular Ito. Am J Physiol Heart Circ-Physiol 2004;286:H602-9. http://doi.org/btzdph

20. Janvier NC, Boyett MR. The role of Na-Ca exchange current in the cardiac action potential. Cardiovasc Res 1996;32:69-84. http://doi.org/d3nn6j

21. Ohno S. Testosterone and cellular response. Birth Defects Orig Artic Ser 1977;13:99-106