INFLUENCE OF TEMPERATURE ON THE AVERAGE VELOCITY OF MUSCLE FATIGUE IN *Rhinella marina* SARTORIUS

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ABSTRACT

Objective: To analyze and compare the effect of temperature on the average velocity of the linear phase and the exponential phase stages produced by periodic stimulation with simple twitching or with tetanus in the toad sartorius. **Methods:** an *in vitro* experimental study with a sample of 46 toad sartorius muscles randomly selected. At the temperatures studied, peak tension produced with two stimulus patterns (twitching and tetanus) were measured until reaching the corresponding muscle fatigue in each case. The tension velocity drop in the linear phase and the exponential phase for each type of fatigue were calculated, and the regression slopes obtained with the Arrhenius equation were compared. **Results:** The temperatures used (1 to 12°C) significantly affected the velocity of fatigue in the stages of linear and exponential phases of both types of fatigue (p <0.05). The function of which the fatigue curves were adjusted to the temperatures used was similar to the function used with the curves at room temperature. When comparing the slopes of Arrhenius regression in the exponential phase and the different stages of the linear phase of each fatigue and between both fatigues, no significant differences were found (p>0.05). **Conclusions:** The temperature significantly affected average development velocity of fatigue in the different phases of the two types of fatigue, but when comparing slopes of most regressions corresponding to Arrhenius there were no significant differences, suggesting that the mechanisms underlying the different stages of fatigue have equal sensitivity to temperature.

Key words: Arrhenius equation, the average velocity of fatigue, muscular fatigue, temperature, tetanus, twitches.

INFLUENCIA DE LA TEMPERATURA EN LA VELOCIDAD PROMEDIO DE LA FATIGA MUSCULAR EN EL SARTORIO DE RHINELLA MARINA

RESUMEN

Objetivos: Analizar y comparar el efecto de la temperatura en la velocidad promedio de las etapas de la fase lineal y de la fase exponencial de la fatiga producida por estimulación periódica con sacudida simple o con tétanos en el sartorio de sapo. **Métodos:** Estudio experimental, *in vitro*; muestra: 46 músculos de sartorio de sapo seleccionados aleatoriamente. A las temperaturas estudiadas, se midió la tensión pico producida con dos patrones de estímulo (sacudida simple o tétanos) hasta llegar en cada caso a un tipo de fatiga muscular, se calculó la

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velocidad de caída de la tensión en las etapas de la fase lineal y en la fase exponencial de cada tipo de fatiga y se compararon las pendientes de las regresiones obtenidas con la ecuación de Arrhenius. **Resultados:** Las temperaturas utilizadas (1 a 12°C) afectaron significativamente ($p<0,05$) la velocidad de la fatiga en las etapas de las fases lineal y exponencial de ambos tipos de fatiga. La función con que se ajustaron las curvas de fatiga a las temperaturas usadas fue similar a la usada con las curvas a temperatura ambiente. Al comparar entre las pendientes de las regresiones de Arrhenius de la fase exponencial y las diferentes etapas de la fase lineal de cada una de las fatigas y entre ambas fatigas, no hubo diferencias significativas ($p>0,05$). **Conclusiones:** La temperatura afectó significativamente la velocidad promedio de desarrollo de la fatiga en las diferentes fases de los dos tipos de fatiga, pero al comparar las pendientes de la mayoría de las regresiones correspondientes de Arrhenius, no se encontraron diferencias significativas, lo cual sugiere que, los mecanismos que subyacen a las diferentes etapas de la fatiga tienen igual sensibilidad a la temperatura.

Palabras clave: fatiga muscular, temperatura, velocidad promedio de fatiga, ecuación de Arrhenius, sacudida, tétanos.

INTRODUCTION

Skeletal muscles are the engines that convert chemical energy into mechanical energy, and can adapt to large demands of work, altering not only their size but also their functional properties. In severe and prolonged activity, muscle performance is deteriorated, both in force generation and shortening velocity, causing muscle fatigue. Although this process is reversible with rest, is complex because it occurs in alternate contraction and relaxation of skeletal muscles and because it depends on: type of muscle fiber, intensity and duration of contractile activity, muscle perfusion and metabolism and animal species in which it occurs. Force alteration in individual muscle fibers in fatigue and sometimes after it, is attributable in part to altered excitation-contraction coupling (1) most likely related to calcium regulation. Although the exact cause of this disorder is still under discussion, contractile activation and/or transient but repeated change of cytoplasmic Ca^{2+} seem to be important factors. There is a growing understanding of the functioning of excitation-contraction coupling and role of Ca^{2+} in this process, knowledge of the organization and function of proteins in the triadic region (2) and contractile structure participation in the process of fatigue (3) should be deepen in the future, but also other possibilities, such as, of which there is also evidence.

Relationship between contractile tension and fatigue during time, it is not a simple function, is composed of a linear phase by stages (4) and an exponential phase valid for both: by single twitch fatigue (STF) or tetanus fatigue (TTF) in *Rhinella marina* (3, 5) toad or *Lithobates pipiens* (6) sartorius and mouse soleus and extensor digitorum longus. Currently due to a lack of clarity regarding the mechanisms involved in muscle fatigue, the study of the parametric behavior of the stages and phases that compose it, after subjecting the muscle to a conditional situation as changing temperature, can be a contribution to the knowledge related to these mechanisms.

This work was based on a principle of chemical kinetics, by which we can say that the velocity of a process that occurs in a medium, is the product of collisions between groups of molecules that are part of it, and its energy exchange at a given temperature (7) and that if change velocity of the process by change the temperature is because its underlying mechanisms have been sensitive to this change (8, 9). This effect is general and

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non-specific, but quantitatively different for each process, and the interpretation is that if a process is sensitive to temperature change is because the molecules involved in it have passed a minimum energy or activation energy (8). Thus, if in the case of ideally identical experiments with muscles at similar fatigability but with different temperatures, similar changes were obtained in the average velocity of fatigue stages (AVF), then it may be inferred that the physical and chemical mechanisms underlying these stages have equal sensitivity to the temperatures studied, the opposite would be if the AVF were different. The relation between the AVF and the temperature was made using the Arrhenius equation since it allows to evaluate the thermal effect on many processes and leads to linear graphs and equations accessible to the analysis in terms of fit and significant differences (10). Since there are parametric differences between fatigue by periodic pattern tetanus (TTF) and periodic single twitch (STF), experiments described were carried out with each pattern or type of fatigue.

As to the comparison between TTF and STF parameters, it involves comparing two different processes, therefore it was more reliable to compare the modification of the same indicator for the two types of fatigue, therefore, intercalated tetanus (TTint.80Hz20p) were introduced as indicator.

MATERIALS AND METHODS

Experimental design: In vitro experimental study, cross-sectional, with several predictor variables and applying a linear regression model. 46 *Rhinella marina* Sartorial muscles were used. Each regression included more than 20 muscles except for two experiments that included 10, all models fulfilled the assumptions for linear regression, and therefore, a smaller sample size could produce a greater variability of the parameter estimates but did not invalidate the results obtained. This study met the requirements of the Act 84 of 1989 (Resolution No. 8430 of 1993) of the Colombian Ministry of Health on animal protection and agreed with the norms of the medical ethics and animal committees of the sponsoring institution and with the Declaration of Helsinki of 1975, updated in 2000. **Muscle preparation and experimental procedure**: Procedures reported (3, 4, 7) include muscle isolation under isometric conditions at optimal length and periodic stimulus. Temperature measurement was made with thermometers (0.1°C) in the chamber where the muscle was placed, which was controlled with a circulating water bath (VWR Circulator Refrigerated Constant Temperature-1155).

Tension recording after stimulation was performed with a Nihon-Kohden electric stimulator, a force transducer, and a Grass polygraph. Prior calibration supra maximum voltages (120%) were used and tension was recorded at room temperature (22°C) and low temperatures. Twitch of 2 ms were produced through a channel every two seconds or tetanus induced by a 80 pulses train of 2ms at 80 Hz every 12s (TT80p80Hz) and through another channel, in all cases intercalating tetanus (TTint) occurred of 20 pulse train at 80 Hz (TT20p80Hz).

In preliminary experiments with different muscles and isolated stimuli for twitch and for tetanus every 30 minutes, there was a change in tension with cooling at temperatures between 1°C and 24°C. Temperature ranges were selected for each case in which tension vs. temperature relationship was linear.

Temperature and muscle were randomly selected for STF and TTF, with cooling temperatures. After obtaining a control tension at room temperature, cooling was produced and twitch stimulation began rapidly for STF or tetanus stimulation for TTF. Simultaneously with the STF stimulus, the TTint were executed every tenth pulse in other channel, if the stimulus was TTF then the TTint was the fourth tetanus with a delay of three seconds. For STF only the last four twitches of each decade between intercalated tetanus were considered, to avoid

transient depression produced by these. 25 sartorial muscle were included for STF and 21 for TTF, each one with it respective TTint, used in three experimental runs.

Tension and fatigue development velocity measurements: Tension was T% relative to the maximum tension at ambient temperature (22°C). In the linear phase peak tensions were included considering linear regression judged by R^2. Similarly stages 1, 2 and 3 reported at room temperature in previous studies (4, 5) were selected, being stage 1 the one that initiated the peak tension fall and stage 3 the one prior to exponential phase, about 50% of the maximum tension fall. Points between these two stages, adjusted by another regression, correspond to stage 2. Early portion of exponential phase was considered for points not belonging to the best fit of the linear phase (R^2), but that had good exponential fit between the tensions corresponding to 50 and 20% of experimental maximum.

A single exponential equation was used to describe this phase: $T_{(t)} = (T_0 - b)e^{-kt} + b$ for which logarithmic form was: $Ln(T\% - b) = LnA - kt$.

$T_{(t)}$ is the peak tension of successive isometric contractions, which is expressed as T%, k is the inverse of the time constant tau (τ) of the tension fall and b is the value which this tension tends asymptotically.

AVF was used as a comparison parameter to evaluate the cooling effect. The AVF value in the linear phase corresponded to the corresponding regression coefficient; these values determined two linear stages (1 and 3) at this phase. In exponential phase, the derivative of each point of fatigue represent the instantaneous velocity (Vi) but as what was required was the AVF in exponential phase, it was calculated by the function integral(11), which, expressed in general, corresponds to the average values of y(x) in a given interval [a, b]: $\bar{y}_{a-b} = \dfrac{1}{b-a}\int_a^b y(x)dx$

and when applied to the early exponential phase of fatigue becomes:

$$\bar{v} = a.v.f. = \frac{1}{t_2 - t_1}\int_{t_1}^{t_2}\frac{dT}{dt}dt = \frac{T(t_2) - T(t_1)}{t_2 - t_1}$$

Velocity of fatigue comparison (Arrhenius equation)

The AVF changes associated with temperature changes between the linear stages and exponential phase of fatigue, was compared using the Arrhenius equation in logarithmic form: $LnK = LnA - \dfrac{Ea}{R}\dfrac{1}{T}$. Natural logarithm of the fall average velocity of tension peak was used in this equation for linear stages (1 and 3), for early exponential and for their respective intercalated tetanus of the STF and TTF in the selected range of temperatures (°K). Arrhenius linear regression slope was named beta (βi) and it measured the fatiguing process sensitivity to temperature change. This parameter was used for the comparison.

Databases and analysis of the results

Data were tabulated with Microsoft Excel and statistical analysis was performed using SPSS. Linear regression analysis was done for the two types of fatigue (two linear stages and early exponential phase), obtaining six regressions. Number (n) of data for each of the regressions was about 20. Assumptions were calculated for all models: linearity (Pearson correlation coefficient r, variance analysis for linear regression and determination coefficient), normality (Kolmorov'Smirnov Test), independence (Durbin Watson (DW) and homoscedasticity (Levene test). Bivariate analysis was made between the variables velocity and temperature to examine their association.

Residuals were examined with SPSS to identify potential outliers based on the criteria of ± 3σ (standard deviation). The errors (residuals) were normally distributed with zero mathematical expectation.

This study met the requirements of the Act 84 of 1989 (Resolution No. 8430 of 1993) of the Colombian Ministry of Health on animal protection.

RESULTS

Preliminary results (three experiments) of temperature effect on single twitch tension as an apparently continuous function and with a maximum around 12°C are shown in Figure 1A. Values were selected in the range of 1 to 9°C because they are in linear relationship, Figure 1B. The effect on tetanus tension is show in Figure 1C and the linear zone is observed in the range of 2.5°C to 12°C (Figure 1D). It was observed that the cooling effect is reversible, since the order in which each muscle was subjected to various temperatures was random.
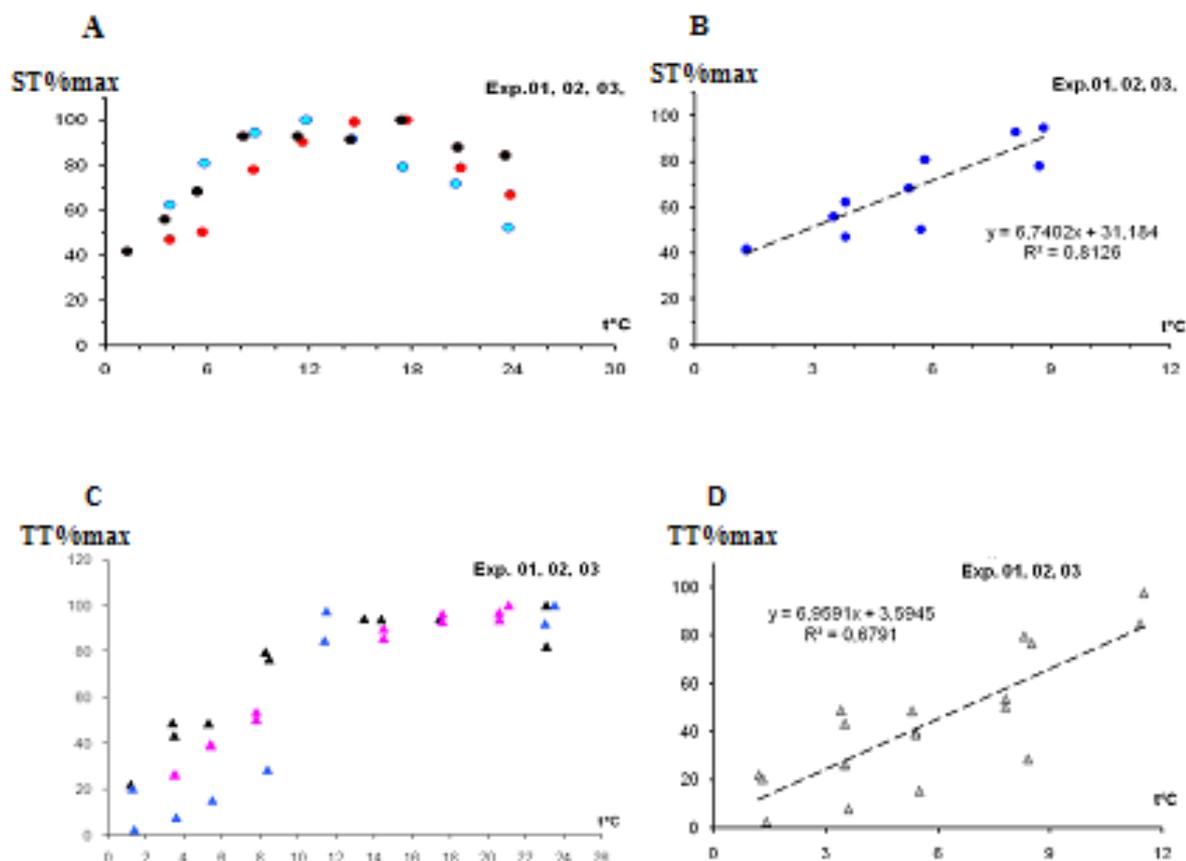

Figure 1. A. Single Twitch (ST) versus temperature (°C) peak tension curve in three muscles. The peak voltage is expressed as a fraction relative to the maximum tension at room temperature for each muscle. B. Temperature range in which there is a linear relationship with the tension peak for ST. C. Tetanus peak tension versus temperature (°C) curve. TT% max: Tension in tetanus as a fraction of maximum tension at room temperature, for each of the three muscles. D. Temperature range in which there is a linear relationship with the peak tension for TT.

Low temperatures fatigue curves

Temporal course of STF and TTF fatigue curves, and their respective intercalated tetanus was qualitatively similar to that observed at room temperature (22°C), with the three successive stages: initial, linear fall of tension and exponential(s) fall(s). The initial stage was not tested because it was not sufficiently reproducible, other phases were analyzed by calculating the parameters described previously. Tension used as 100% in each curve was the initial value of the maximum tension at room temperature (22°C).

Single Twitch fatigue

In the linear phase of this fatigue, "Stage 1" and "Stage 3" appear, but the stage 2 was virtually absent (Figure 2). The values of AVF represent the linear fit slopes of each stage for twitch and intercalated tetanus. In phase "early exponential" there were good fit according to the R^2. The average velocity was obtained with the parameters (k, тau, b) for twitch and intercalated tetanus.
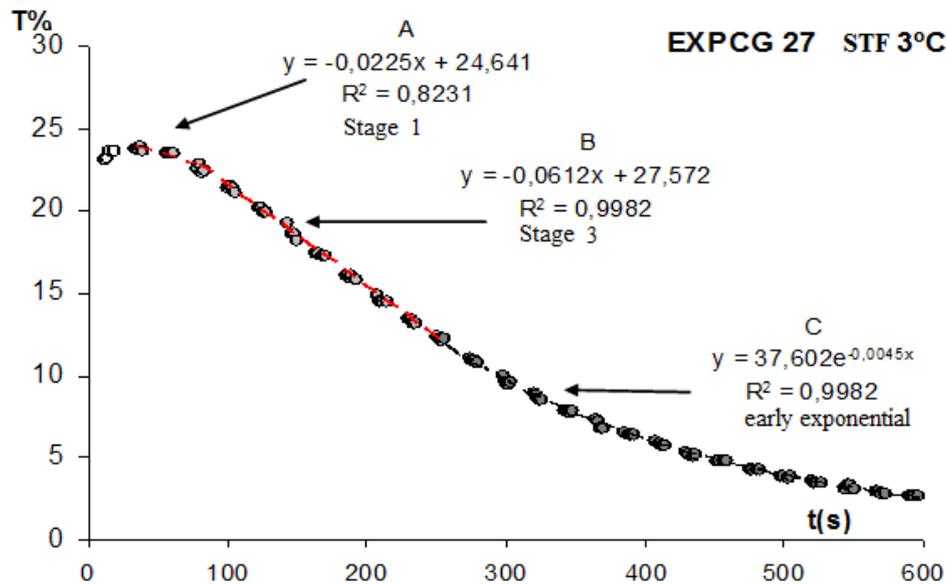

Figure 2. Single Twitch fatigue (STF) curves phases and stages at low temperature (3°C): Initial: Slight potentiation, linear falls phase with linear stages (A, B) and early exponential phase (C). The points on the curve correspond only to the last four twitches for each decade between intercalated tetanus.

Periodic Tetanus Fatigue

Slope calculation or AVF of the linear phase of the two types of tetanus (TT80p and TTint20) of this fatigue (TTF) was easy in stage 3, decreased sufficiently in stage 1 and it was not possible at stage 2. Good fit was achieved with an R^2 close to 0.9. After phase 3 continued the exponential decay phase between the voltages of 50 to 20% of the maximum peak voltage experimental ("early exponential") had good fit ($R^2 > 0.9$) as a single exponential. As in STF, the tension used was T%. The values of the corresponding parameters (k, т, and b) and the fav for the intercalated tetanus and TT80p as well as corresponding linear fit values were considered.

Effects of temperature on fatigue velocity (Arrhenius equation)

In exploring with the Arrhenius equation of fatiguing process sensitivity (β1) to temperature change in stage 1 and 3 and the early exponential of STF and TTF experiments, and their respective intercalated tetanus was found that of the eleven simple linear regressions ten showed a statistically significant linear relationship ($p < 0.05$) with r between 0.532 and 0.796, and complying with all statistical assumptions, with exception of sector TTF-TT20-S1 ($p = 0.349$, n = 6) for which the number of data was low (see Figures 3 and 4). Bivariate analysis (ANOVA) showed also that in the ten linear regression models, there is a statistically significant association between the temperature ($1/ T°$) and the Ln AVF (average velocity fatigue Logarithm).

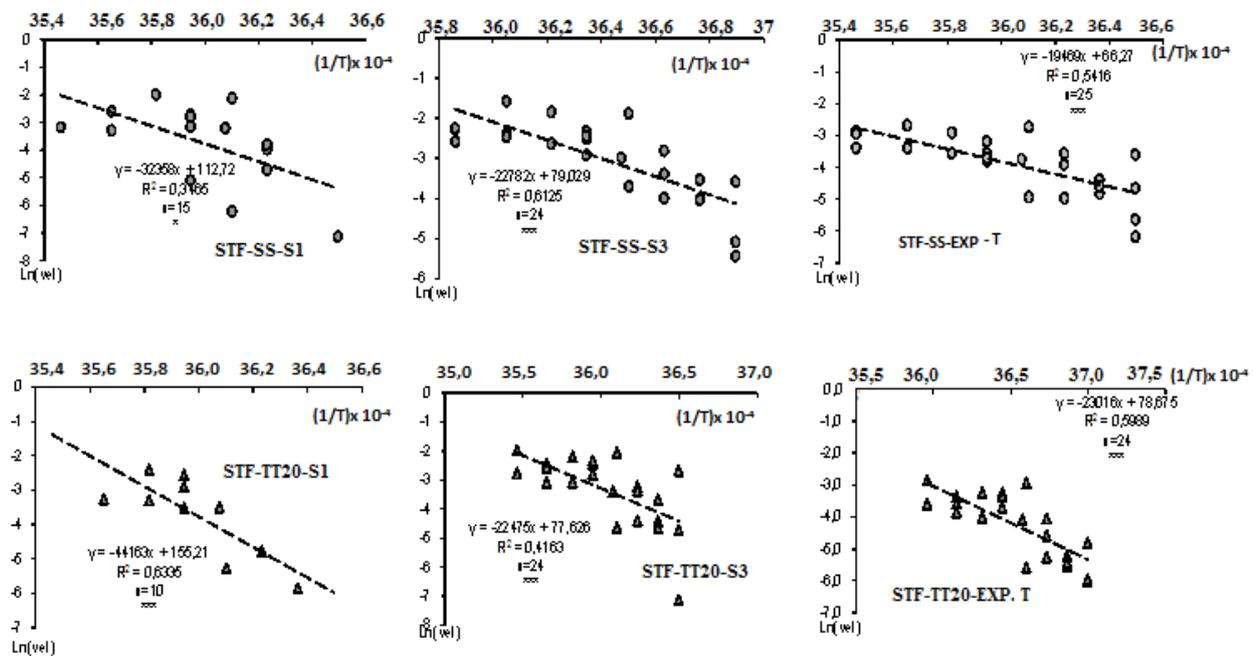## ARRHENIUS RELATION FOR SINGLE TWICH FATIGUE

Figure 3. Arrhenius graphs for fatigue: Stage 1 (S1), Stage 3 (S3) and early exponential (Early Exp.). A. Arrhenius graphs for twitch fatigue (STF). B. Arrhenius graphs for intercalated tetanus fatigue (TF-TT20). The coordinates are: X = 1/ Temperatura (° K), Y = natural logarithm of velocity (Ln(vel)), N = number of data, statistical significance: (*) = $p < 0.05$, (**) = $p < 0.005$, (***) = $p < 0.001$.

Comparison of slopes with the Arrhenius model

When making comparisons of slopes (βi) between different stages, intra-stimulus, we found no statistically significant differences. Significant comparisons were between TF-TT20-S1 and TF-TT20-S3 and between TF-TT20-S1 and TF-TT20Exp.Tp, but was not very reliable, since the TF-TT20-S1 has several data under 20 (n = 10). Comparison between the slopes of the same stage but with different stimulus patterns, show no significant differences between them for the linear phase, but for the early exponential phase, there was a marginally significant difference ($p = 0.052$).
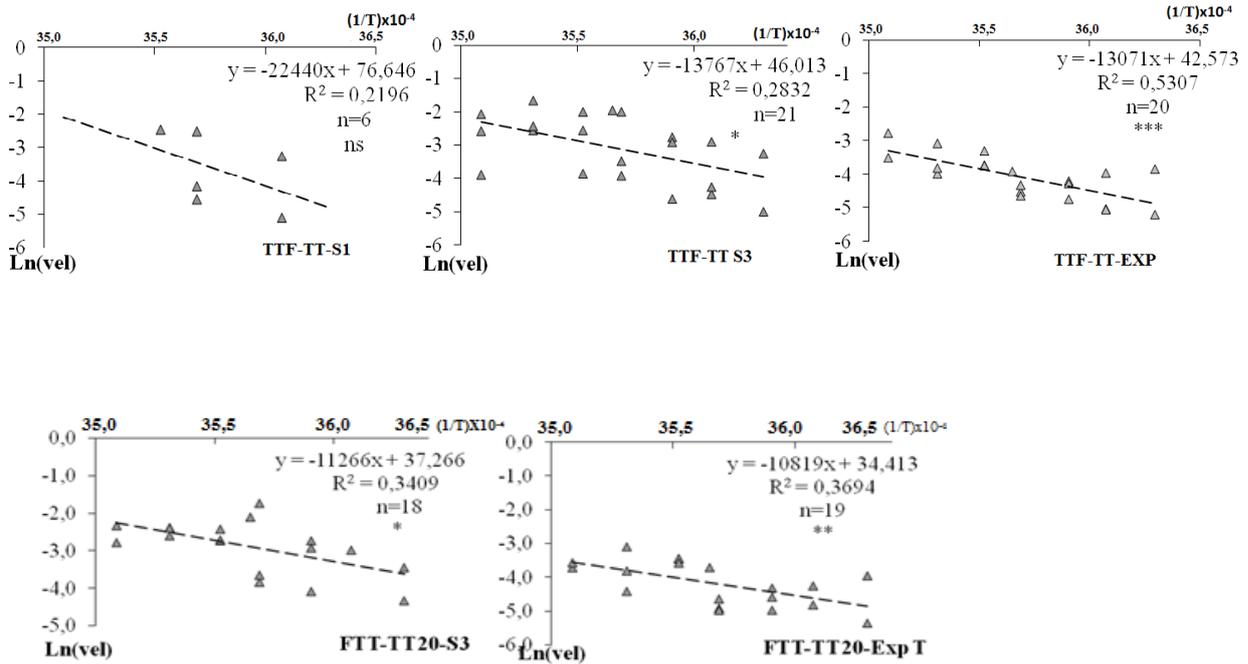
Figure 4. A. Arrhenius graphs for Tetanus Fatigue (TTF): Sector 1 (S1), Sector 3 (S3) and early exponential (Early Exp.). B. Arrhenius graphs for intercalated Fatigue Tetanus (TTF-TT20). The coordinates are: X = 1/Temperature (° K), n = number of data, Y = natural logarithm of velocity (Ln(vel)) Statistical significance: (ns) = not significant, (*) = p <0 05, (**) = p <0.005, (***) = p <0.001

DISCUSSION

The main objective in this work was to explore under identical conditions, the sensitivity of the velocity of fatigue, in toad sartorial, when cooling medium. Velocity difference between the stages of the linear phase or exponential phase of fatigue (STF and TTF) represent differences in the sensitivity of the reactions that support them. TTF and STF linear and exponential fall curves at low temperature, were similar to those observed at room temperature for *Rhinella marina* (3), frog (13) and iguana. Different parameters have been used in the study of fatigue (3, 5, 12). In this study analysis, a new parameter that was the AVF was used, which represents the rate at which the force falls at each stage of fatigue, enabling comparison. This parameter was used in the Arrhenius equation (8, 10), given that the hypothesis is supported by fatigue as a physicochemical change of contractile structure (14, 15) whose behavior can be altered with cooling. Linear regression analysis between Ln AVF of each stage and temperature with the Arrhenius equation, satisfied all linear regression assumptions and fit better than direct regressions between AVF vs. temperature, this can be seen in Figures 3 and 4 in which appears the coefficient of determination (R^2) with statistical significance at probability <0.05 and in 90% of the regressions.

Comparison of most of Arrhenius regressions slopes (β1) were not significantly different between the stages of the same fatigue and between homologous stages of the two fatigues, suggesting that the sensitivity of the physicochemical mechanisms underlying these fatigue stages are equal.

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Three significant differences between interstimulus and intrastimulus slopes (βi) were found, but they were not very reliable since two of them had a low n (n <20) and in the other there was a marginally significant difference.

Despite the good fit and the results found using the Arrhenius equation, the average R^2 values was: 0.502 for STF and 0.344 for TTF, implying the presence of other variables than temperature (difference between animals, cross sectional area and muscle experimental order) to which the fatigue velocity is sensitive.

With cooling, besides decreasing peak tension it was seen (data not shown) that contraction velocity was smaller but for relaxation was even smaller, resulting in a asymmetrical register, while at room temperature, absolute values of these velocities were higher and symmetric. These changes with cooling have been reported by other authors in isolated fiber of amphibians (7, 16) and frog and toad muscles at temperatures between 0 and 20°C (17). Muscle tension response to cooling may vary depending on the species, the type of fiber and the type of stimulus (18, 19).

It is important to clarify issues about certain methodological aspects: maximum of each curve T% was used with respect to the reference temperature of 22°C to avoid matching results at different temperatures when normalized. In the comparative study of temperature effect on periodic twitch STF and periodic tetanus TTF, previously designed protocols were used to produce similar degree and time course fatigue to room temperature (4). Short intercalated tetanus could be used as indicators for each type of fatigue (STF and TTF) because they had not changed during each interval.

AVF is a useful parameter to evaluate and compare the effect of any factor that alters peak tension fall in fatigue stages. Temperature significantly affect the average velocity of fatigue development in different phases of two kinds of fatigue, but when corresponding Arrhenius regressions slopes were compared most differences were not significant, so it can be stated that the mechanisms underlying the two phases of fatigue have a similar sensitivity to temperature changes.

It seems that the speed changes in fatigue must do with the kinetics of cross bridges of muscle system. This has been observed with changes in phosphates and changes in temperature (11, 20, 21).

CONCLUSIONS

Average velocity of fatigue was affected significantly by temperature, in the different phases of the two types of fatigue, but when comparing slopes of most corresponding Arrhenius regressions there were no significant differences, suggesting that the mechanisms that underlie different stages of fatigue have equal sensitivity to temperature.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

There was no conflict of interest with the institution or research group that supported the development of this project.

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