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The Effect of Temperature on Canine Papillary Muscle

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Summary. Measurements of the total duration, t_A, of the action potential for canine papillary muscle in the temperature range 25–45° C are shown to follow the law

$$t_A = \tau \exp(Q/k_B T),$$

with τ and the activation energy Q independent of temperature, T, and k_B equal to Boltzmann's constant. The value obtained for $Q = 0.65 \pm 0.10$ eV.

Results are also presented for the temperature dependence of points $V(x)$ on the action potential curve, where x is the percentage of repolarisation within the range $30\% < x < 100\%$.

Key words: Action potential – Temperature – Heart – Activation Energy – Duration.

Introduction

A number of studies have already investigated the effect of temperature on the action potential of single cardiac cells (Woodbury, Hecht and Christophersen, 1951; Coraboeuf and Weidman, 1954; Fingle, Woodbury and Hecht, 1952; Heintzen, 1954). We have found no previous study on canine papillary muscle although an investigation was carried out on dog Purkinje fibre (Trautwein, Cottstein and Federschmidt, 1953) which indicated the importance of stimulation rate when analysing the effect of temperature on duration.

This latter investigation in common with other studies (Heintzen, 1954) states Q_{10} values, whereas we have favoured fitting the results to an Arrhenius equation, leading to a rate limiting activation energy for the process. Our study is comparable to the analysis of the effect of temperature on frog ventricle undertaken by Woodbury et al. (1951). In the present study the action potential from single cells was recorded over a temperature range of 25–45° C. An Arrhenius plot was then made throughout the repolarisation phase of the action potential. We were particularly

interested to determine of the activation energy could be used as a parameter for characterising the potency of antiarrhythmic drugs.

The measurements presented here demonstrate that the total duration of the action potential in canine papillary muscle is determined by a process with a rate limiting activation energy Q ($\sim$ 0.6 eV), and the change in activation energy which occurs as the cell repolarises after the initial spike is also considered.

Experimental Method

Papillary muscle was obtained from healthy mongrel dogs of either sex which were anaesthetised with sodium pentabarbitone, 30 mg/kg body weight. Ventilation with 50% oxygen via an intra-tracheal cannula was applied prior to opening the chest and removing the beating heart. The muscle was then excised and placed in oxygenated physiological solution at 37.0 $\pm$ 1° C. Specimens of muscle, approximately 5 mm long and not more than 1 mm thick, were mounted in the tissue bath and left beating for 1 h before measurements began. The tissue bathing solution consisted of 130 mM NaCl, 4.0 mM KCl, 22.0 mM $NaHCO_3$, 0.435 mM NaH_2PO_4 0.2 H_2O, 0.4 mM $MgCl_2$ 0.6 H_2O, 1.8 mM $CaCl_2$ 0.6 H_2O and 5.56 mM dextrose. Solutions were equilibrated with a gas mixture of 5% CO_2/95% O_2.

In order to perform an experiment on single papillary muscle fibres we found a flexible electrode suspension, similar to that described by Woodbury and Brady (1956) to be the easiest to use. Owing to the poor electrical stability associated with tunsten wire, a flexible spring was produced from 0.25 mm diameter silver wire, one end of which was chlorided for insertion into glass micropipettes filled with 3.0 M potassium chloride solution.

Early experiments were performed using a tissue bath and oxygenator fully described by Coltart and Meldrum (1971), but in later experiments this was improved by gassing the tissue bathing solution in a separate reservoir and then pumping it, via a heat exchanger, through the bath containing the tissue. A major advantage of this system is that it removes interaction between the solution flow rate and the O_2/CO_2 bubble rate. The heat exchanger allows the temperature of the physiological solution

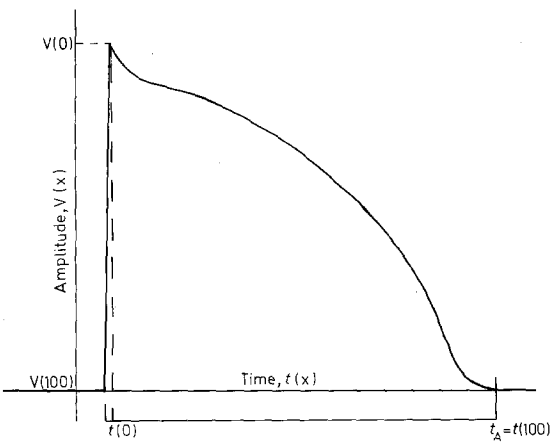

Fig. 1. A typical action potential recorded during an experiment, showing the relationship between $V(x)$, $t(x)$, t_A and the total duration

to be varied rapidly, so that measurements may be made first with the temperature falling until the cell stops firing and then again as the temperature is raised.

In all experiments the tissue was stimulated by a pair of platinum electrodes using a square pulse derived from a Grass stimulator (SD9). The magnitude of the pulse was set at twice the threshold voltage and the pulse frequency was 30 beats/min. An action potential recorded from a typical experiment is shown in Figure 1. We would now recommend the addition of sodium acetate and insulin to the bathing solution to aid the maintenance of cell metabolism and also, stimulation of the tissue at lower frequencies, so that energy requirements are minimised.

Results

a) Total Duration of Action Potential

Figure 2 shows that the duration, t_A, of the action potential from canine papillary muscle can be related to temperature T by,

$$t_A = \tau \exp (Q/k_B T) \tag{1}$$

where τ and Q are constant over the temperature range 25–45° C and k_B = Boltzmann's constant. τ has dimensions of time, and Q is interpreted as a characteristic activation energy.

Equation (1) has been found to apply for a total of 14 cells from 12 separate tissue specimens. The mean value of Q was found to be 0.65 ± 0.1 eV. In two experiments at 12 beats/min, and with the addition of insulin and sodium acetate to the bathing solution, the activation energies were found to fall within the same range.

b) Duration for a Given Percentage Repolarisation

In view of the excellent fit to the total duration t_A as a function of $1/T$ by Equation (1), we proceeded to determine an activation energy associated with different parts of the repolarisation phase.

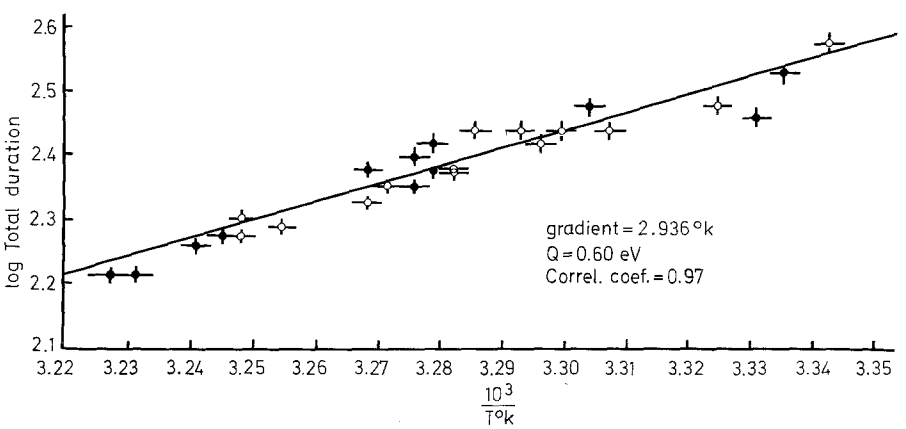

Fig. 2. $\text{Log}_{10} t_A$ versus $10^3/T$ plotted for a single canine papillary muscle cell. —●— has been plotted for increasing temperature; —○— for decreasing temperature

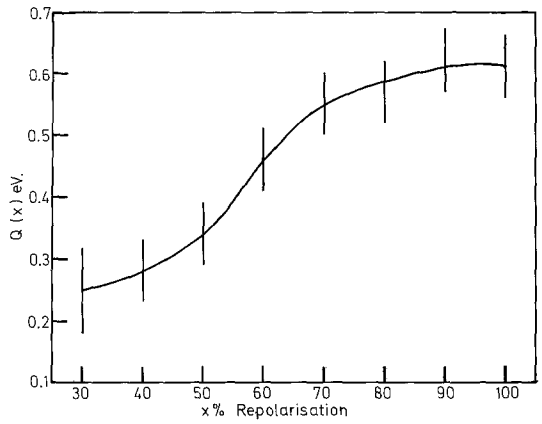

Fig. 3. Variation of the activation energy $Q(x)$ as a function of x% repolarisation. The error bar is derived from the standard error on the slope, $Q(x)/k_BT$, of the regression line

Writing a generalisation of (1) as

$$t(x) = \tau(x) \exp (Q(x)/k_BT) \tag{2}$$

Where x is the percentage repolarisation, and the generalised activation energy $Q(x)$ evidently reduces when $x = 100\%$ to that in Equation (1).

It is found from our measurements that the temperature dependence of the action potential curve (for $x > 30\%$) is well described by Equation (2), the quantity $Q(x)$ varying as in Figure 3. It can be seen from this plot that for $x > 60\%$ the activation energy $Q(x)$ reaches an equilibrium value ~ 0.6 eV.

Discussion

We have established Equation (1) experimentally and determined Q as (0.65 ± 0.10) eV in canine papillary muscle. The activation energies we obtained are comparable to those obtained by Woodbury et al. (1951) for the frog ventricle. Using the nomenclature described by Cranefield and Hoffman (1958); for phase 2 we obtained a value of 0.25 eV, and for phase 3 we obtained 0.60 eV. The equivalent values quoted for frog ventricle were 0.4 eV and 0.5 eV respectively. In the analysis for the frog ventricle, the change in the gradient of each phase of the action potential was used to obtain the activation energy, and therefore these results represent an average for the two phases.

One possible rate limiting mechanism with an activation energy of this order, is ionic diffusion. Support for such a model is found by considering the effect on the action potential of substituting strontium for calcium in the tissue bathing solution. When strontium is substituted for calcium in the tissue bathing solution, the duration of the action potential from the SA node is found to be increased (Toda, 1971). A diffusion model predicts that the duration should be increased by the square root of the mass ratio, that is (M_{Sr}/M_{Ca}). This yields a factor of 1.48, whereas an increase by

a factor of about 2.1 is found by Toda (1971). However, Toda also reported that strontium alters the pacemaker frequency. In the calcium bathing solution the mean cycle length was 660 ms, whereas in the case of strontium the length was 918 ms. Carmeliet and Lacquet (1956) found that duration was empirally related to cycle length by

$$t_A = t_\infty (1 - \exp(-\alpha c) \tag{3}$$

which to first order gives $t_A = (t_\infty \alpha)c$ where $(t_\infty \alpha)$ is constant and c is the cycle length. $(t_\infty \alpha)$ obtained from Toda's data can be used to predict the change in duration resulting from a change in cycle length. When this is done, the duration of the action potential in the strontium bathing solution is calculated to be $(M_{Sr}/M_{Ca})^{1/2} \cdot (t_\infty \alpha)c = 397$ ms, which compares very well with the observed value of 404 ms.

This interpretation is in no way inconsistent with other work in this field. We are not suggesting that Ca^{++} diffusion is the mechanism responsible for generating the action potential, our study has been entirely concerned with the factor controlling the duration. In a recent paper further evidence has been presented to support the central role of Ca^{++} in determining the duration of the action potential (Isenberg, 1975).

We have not attempted here a detailed analysis of the generalised activation $Q(x)$. However, we feel that the considerable change in the activation energy (a factor of about 2–3) during the repolarisation phase is of physical significance, and hope to present a discussion of this, together with the effect of the drugs, at a later date.

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