

THE EFFECT OF TEMPERATURE ON THE TENSION RESPONSES OF THE ANTERIOR BYSSAL RETRACTOR MUSCLE (ABRM) OF *MYTILUS EDULIS*

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SUMMARY

The effect of ambient temperature on the response of the ABRM of *Mytilus edulis* to acetylcholine and 5-hydroxytryptamine has been examined. As the ambient temperature was increased, the latent period and the maximum tension developed decreased while the rate of tension development and the rate of relaxation increased. The relationship between temperature and the rate of tension development showed three distinct linear phases from 2-25, 25-35 and 35-45 °C. The reduction in peak tension with temperature could also be resolved into three portions from 2-25, 25-40 and above 40 °C. As the temperature was increased above approximately 27 °C the rate of relaxation in the absence of 5-HT approached the rate of relaxation in the presence of 5-HT. The significance of these results and possible explanations for them are considered.

INTRODUCTION

It has long been known that muscle tension is temperature-dependent. A negative temperature coefficient, where the tension produced in response to a given stimulus decreases with increasing temperature, is shown by some whole muscle preparations (Ranatunga, 1977; Close & Hoh, 1968; Hill, 1951; Doi, 1920; Edman, Mattiazzi & Nilson, 1974; Apter, 1972; Mashima & Matsumura, 1964; Kaufmann & Fleckenstein, 1965; Kelly & Fry, 1958), and isolated fast twitch fibres (Buller, Ranatunga & Smith, 1968).

The converse, a positive temperature coefficient, is observed in some smooth muscles (Csapo, 1954) and slow twitch fibres (Buller *et al.* 1968; Close & Hoh, 1968).

Since these studies have been largely confined to vertebrate muscle, the object of the present investigation was to examine the tension-temperature relationship in a selected invertebrate muscle.

The invertebrate muscle chosen for study was the anterior byssal retractor muscle (ABRM) of *Mytilus edulis*. There were two reasons for this choice; firstly, Twarog (1967*b*) mentions that some responses of the ABRM appear temperature sensitive and, secondly, as a preparation it has been studied extensively since 1937 (Winton,

1937; Twarog, 1954, 1960, 1967*a, b, c*, 1976, 1977; Twarog & Cole, 1972; Twarog, Dewey & Hidaka, 1973; Twarog & Hidaka, 1971; Twarog & Muneoka, 1973; Lowy & Millman, 1963; Gilloteaux & Baguet, 1977; Cornelius & Lowy, 1978) having fascinated workers with its ability to hold tension for prolonged periods of time without apparent active state activity (Johnson & Twarog, 1960) or energy expenditure (Baguet & Gillis, 1968).

MATERIALS AND METHODS

Animals

Specimens of *Mytilus edulis* were obtained from the Gatty Marine laboratory, University of St Andrews. They were stored either in aerated sea water, or in a refrigerator at 4 °C for up to 4 days before use.

Dissection

The ABRM was dissected using the method described by Twarog (1954). The muscle was stripped of all connective and nervous tissue and trimmed to obtain a bundle of fibres of approximately 1 mm. A hook was tied around its base, anterior to the foot and byssal organ.

All experiments were conducted at a standard muscle length, 80% of the *in situ* length (L_0).

Apparatus

The muscle was suspended vertically. A small piece of shell left attached to the muscle during dissection held one end of the muscle in a glass stirrup and a silver chain attached the other end to an E and M Linear Core Isometric Transducer (range 0–1 kg). The transducer was mounted on an adjustable micrometer, which allowed the muscle length to be varied to within 0.01 mm. The whole unit was supported on a moveable stand, allowing it to be raised and lowered into solutions of varying temperatures within 5–7 s.

For the purpose of these experiments, it was essential to maintain the temperature of the test solution within ± 1 °C of a given value between 2 and 40 °C for at least 15 min. This was achieved by placing the containers holding the test solutions into 'wells' cut into a polystyrene block. Polystyrene has a low thermal conductivity and tests showed that the change of temperature was less than 0.01 °C min⁻¹ even when the initial temperature was as much as 20 °C below ambient temperature. The temperature was monitored by using a thermistor probe, and previous work had shown that 90% equilibration occurred throughout the muscle within 7–10 s of immersion (Woods, 1974).

Procedure

After dissection the muscle was placed in the experimental chamber and allowed to equilibrate in a sea water solution for at least 1 h before experimentation commenced. Tension was induced by the application of 10⁻³ M acetylcholine chloride (ACh) for 1 min. At this concentration a reproducible tension, approximately 90–100%

Maximum, was obtained and repeated contractures could be obtained without any visible adverse effects on the muscle. Tension was abolished with 10^{-6} M 5-hydroxytryptamine creatine sulphate complex (5-HT). These drugs were either made up directly in sea water or as concentrated stock solutions in distilled water which were diluted as required in the experimental solution so the ionic strength of the sea water was not reduced by more than 1%. The concentrations of 5-HT and ACh were only varied in some control experiments.

To initiate phasic contractions, a high K^+ artificial sea water (ASW) solution was used, in which 320 mM-NaCl was replaced by KCl (Nagai & Hagiwara, 1970).

Control experiments showed that in the majority of cases a reproducible tension response could be obtained at temperatures up to approximately 35 °C. However, at temperatures above 25 °C muscles were stimulated only once since at, and above these temperatures an irreversible effect on muscle proteins could not be excluded.

Solutions

Fresh or artificial sea water was used in all experiments. No difference in result was detected between the two. The ASW used was that of Nagai & Hagiwara (1970): NaCl 450 mM, KCl 10 mM, $CaCl_2$ 10 mM, $MgSO_4$ 51 mM, 50 mM Tris (hydroxymethyl) aminomethane (pH 7.3–7.4).

Recording

Tension responses were recorded on a Bryans (27000) chart recorder.

Analysis of tension responses

Unless otherwise stated, tension is expressed in absolute terms – kg/cm^2 . Muscle length is expressed as a multiple of the *in situ* length (L_0). The parameter dP/dT (the rate of tension development) was obtained by drawing a tangent to the tension increment and calculating the slope; dP/dT is expressed in $kg/cm^2/s$.

Statistics

A student *t*-test was used for statistical evaluation of the results which are expressed as the mean \pm 1 standard error of the mean. Regression analysis was undertaken in appropriate instances to estimate the statistical significance of the results.

Reagents

The sources of drugs used in this study are as follows: acetylcholine chloride; 5-hydroxytryptamine creatine sulphate complex; Sigma Chemical Company, St Louis, U.S.A.

Unless otherwise stated all reagents used were analytical grade.

RESULTS

Responses of the ACh-induced tension to changing temperature

Tension development resulting from the addition of ACh is slow, the average time to P_{\max} recorded in this study being 32.6 ± 9 s at 20°C and 142 ± 6 s at 2°C ; relaxation is not spontaneous but takes hours or even days unless a relaxant, such as 5-HT or Dopamine, is applied (Twarog, 1954, 1960, 1967*b*). These values are several orders of magnitude greater than in frog skeletal muscle where, at 20°C , an average time to P_{\max} is 50 ms and relaxation may be complete in 100 ms.

All aspects of the ACh contraction-relaxation cycle examined in this study were found to be temperature-dependent. These include the latent period, the rate of tension development, peak tension, and the relaxation rate in the presence and absence of 5-HT.

The latent period

The latent period (LP) was found to decrease from 7.3 ± 1.3 s at 2°C to 2 ± 0.1 s at 20°C (Q_{10} 1.73 ± 0.05). This can be compared to rat portal smooth muscle where the Q_{10} for the LP is 1.63 (Peiper, Laven & Ehl, 1975).

Rate of tension development

The log-linear plot of dP/dT against temperature shows three distinct phases, from $2-25^\circ$, $25-35^\circ$ and $35-45^\circ\text{C}$, with respective slopes of 0.0032 ± 0.0014 ; 0.035 ± 0.004 and 0.000 ± 0.0023 .

Peak tension

Peak tension, in response to 10^{-3} M-ACh, is inversely related to temperature (i.e. the muscle shows a negative temperature coefficient). The reduction in P_{\max} associated with an increase in temperature can also be resolved into three portions; the first extends from $2-25^\circ\text{C}$ where the reduction in P_{\max} is of the order of 0.11 ± 0.003 kg/ $^\circ\text{C}$, the second from $25-40^\circ\text{C}$ (0.06 ± 0.006 kg/ $^\circ\text{C}$), and the third above 40°C (0.18 ± 0.002 kg/ $^\circ\text{C}$).

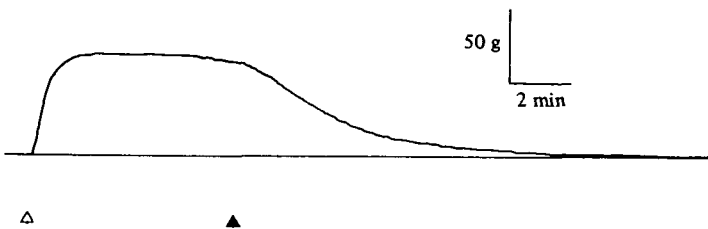


Fig. 1. Typical ACh-induced contraction relaxation cycle. Tension was initiated by the addition of 10^{-3} M-ACh (Δ) for 1 min at 20°C ; and abolished by the application of 10^{-6} M-5-HT (\blacktriangle) also at 20°C . Muscle length was $0.8 L_0$.

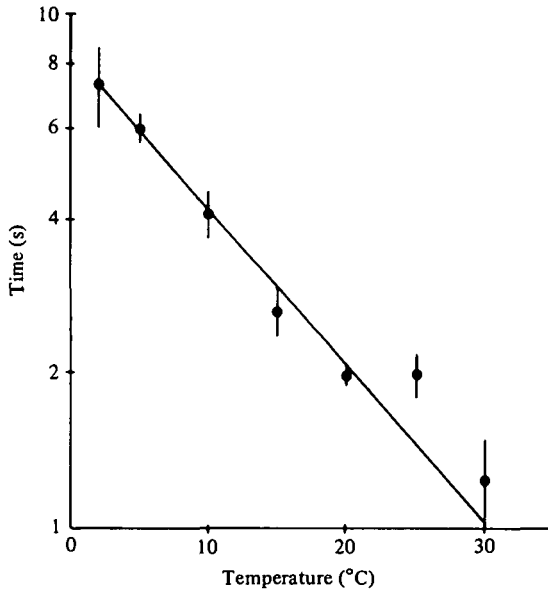


Fig. 2. Latent period-temperature relationship. The log-linear plot of latent-period duration with temperature shows that the latent period (LP) after stimulation with 10^{-8} M-ACh decreases with increasing temperature, from 7.3 ± 1.3 s at 2°C to 1.25 ± 0.25 s at 30°C . Each point represents the mean of seven observations, and the vertical bars the standard error of the mean. The line was fitted by eye. Muscle length: $0.8 L_0$.

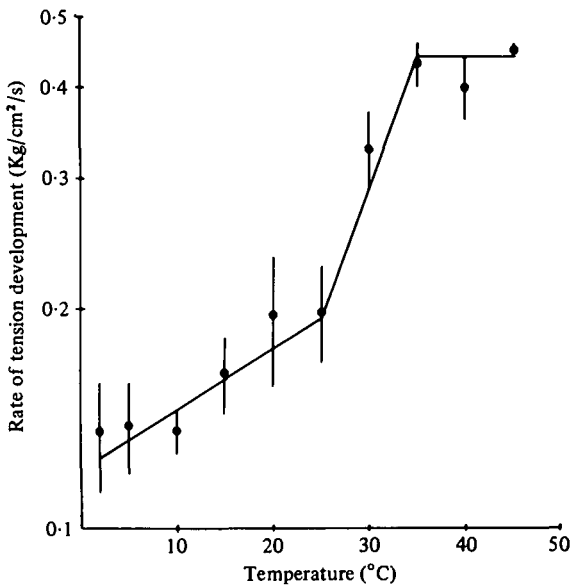


Fig. 3. Relationship between temperature and ACh-induced tension development. The rate of ACh-induced tension development increases with increasing temperature. The log-linear plot shows three distinct phases, between 2 and 25°C , 25 and 35°C and 35 and 45°C . Each point represents the mean of six observations and the vertical bars the standard error of the mean. Muscle length was $0.8 L_0$.

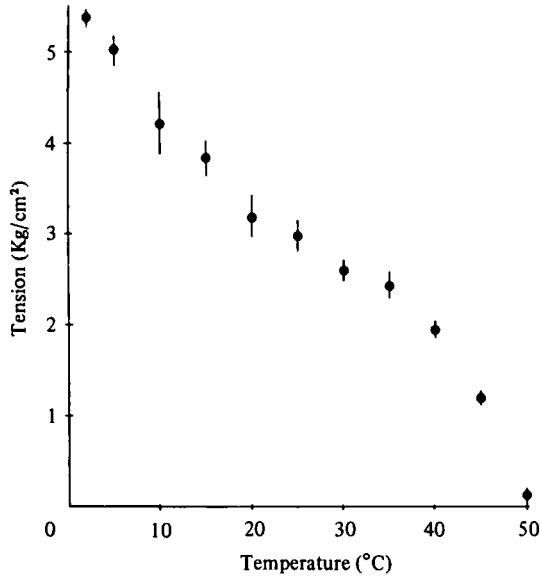


Fig. 4. Relationship between ACh-induced tension and temperature. Peak ACh-induced tension decreases as the temperature is increased, from 5.39 ± 0.12 kg/cm² at 2 °C to 1.2 ± 0.049 kg/cm² at 45 °C. Each point represents the mean of seven observations and the vertical bars the standard error of the mean. Muscle length: $0.8 L_0$.

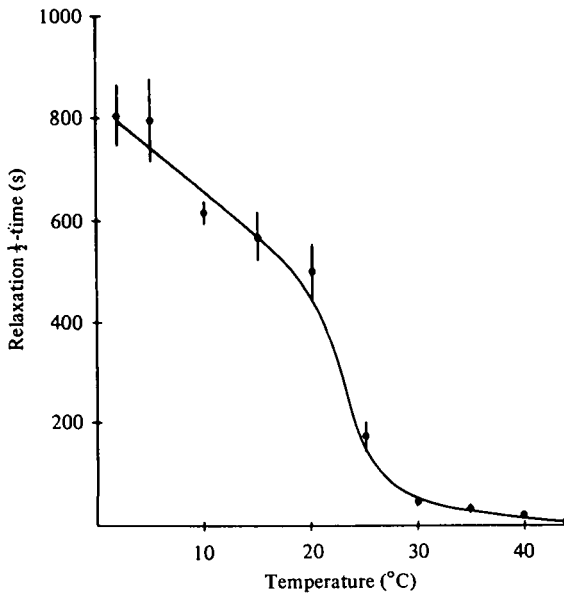


Fig. 5. The effect of temperature on the relaxation half-time ($t_{\frac{1}{2}}$) of the ACh-induced tension. The time to 50% relaxation ($t_{\frac{1}{2}}$) after the addition of 10^{-8} M-ACh decreases with increasing temperature, from 805 ± 60 s at 2 °C to 9 ± 1.2 s at 45 °C. Each point represents the mean of six observations and the vertical bars the standard error of the mean. Muscle length: $0.8 L_0$.

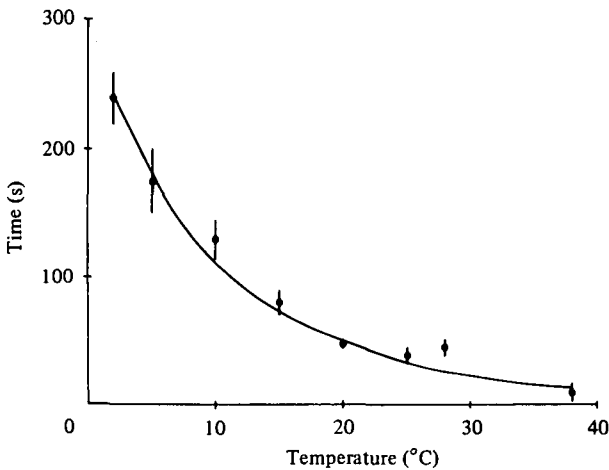


Fig. 6. The effect of temperature on 5-HT-induced relaxation. The time to 50% relaxation ($t_{\frac{1}{2}}$) response to 10^{-6} M-5-HT decreases as temperature increases, from 240 ± 20 s at 2 °C to 10 ± 4 s at 40 °C. Each point represents the mean of six observations, and the vertical bars the standard error of the mean. Muscle length: $0.8 L_0$.

Relaxation

In the absence of 5-HT, relaxation from an ACh-induced contracture occurs slowly and shows a positive temperature relationship. At 2 °C the mean relaxation half-time ($t_{\frac{1}{2}}$) was 805 ± 60 s, and at 20 °C it had decreased to 500 ± 62 s. As the temperature of the contracture-relaxation cycle was increased from 20 to 30 °C the relaxation half-time decreased tenfold, from 500 s at the lower temperature to 50 s at the upper temperature. When the relaxation $t_{\frac{1}{2}}$ becomes faster than approximately 200 s the muscle is said to relax spontaneously. Above 30 °C the relaxation half-time decreased by 2.5 s/°C.

5-HT-induced relaxation

At temperatures below 30 °C, 5-HT application markedly increased the relaxation rate; the half-time of 5-HT relaxation is 240 ± 20 s at 2 °C, and 43.6 ± 4 s at 20 °C ($Q_{10} 3.5$ 2–25 °C). At temperatures above 27 ± 3 °C the relaxation $t_{\frac{1}{2}}$ of 5-HT and spontaneous relaxation approach one another (5-HT $t_{\frac{1}{2}}$ at 30 °C is 43.6 ± 2.6 s; spontaneous relaxation $t_{\frac{1}{2}}$ is 48.8 ± 5 s).

K-contractures

It may also be noted here that K-contractures initiated in the ABRM also show a negative temperature coefficient. Over the range 20–2 °C the size of the response approximately doubled in association with a decrease in the rate of tension development and relaxation. In contrast to the findings reported here Caputo (1972) found that K-contractures in the frog sartorius were greatly prolonged at lower temperatures, but maximum contracture tension decreased by 15% between 20 and 3 °C.

DISCUSSION

ACh response in relation to temperature

One consistent feature of the ABRM which has emerged from this study is that, like fast twitch fibres (Buller *et al.* 1968) it shows a negative temperature coefficient (i.e. as the temperature was decreased the tension produced during an ACh-induced contracture increased). This property has been noted in other preparations, e.g. rat extensor digitorum, tortoise iliotibialis, frog sartorius and gastrocnemius, rat triceps surae, rabbit papillary and ventricular muscle and in cat iris sphincter (Ranatunga, 1977; Close & Hoh, 1968; Hill, 1951; Doi, 1920; Edman *et al.* 1974; Apter, 1972; Mashima & Matsumura, 1964; Kaufmann & Fleckenstein, 1965; Kelly & Fry, 1958). The converse, a positive temperature coefficient, is observed in slow twitch fibres, e.g. soleus (Buller *et al.* 1968; Close & Hoh, 1968), and in some smooth muscles, e.g. uterine (Csapo, 1954).

Various proposals have been advanced to explain the inverse temperature-tension relationship of skeletal muscle. A. V. Hill (1951) suggested that final twitch tension depends on the balance between two opposing reactions: the internal shortening of the contractile element, and the decay of activity. Normally, the tension produced during a muscle twitch is less than that of a tetanus, and this has been attributed to the rapid onset of relaxation allowing insufficient time for internal shortening to be completed. Hill suggested that the relaxation process is more temperature-sensitive than the activation process, so that, as the temperature is lowered, the former rate is reduced more than the latter and total tension is increased. This hypothesis was based on earlier observations of Hartree & Hill (1921), who found that the temperature coefficient of the rate of rise of twitch tension was 2.5, while that of relaxation was 3.6. In this report the value for the rate of development of ACh tension in the ABRM was 1.2 from 0 to 25 °C and 2.15 from 25 to 35 °C, while that for relaxation was 1.2 from 2 to 20 °C and 3.5 from 20 to 30 °C, suggesting that in this particular model, over the temperature range 0–20 °C, the hypothesis of Hill does not hold. However, other theories may be equally applicable. One suggests that decreasing the temperature prolongs the duration of the action potential (Ward & Thesleff, 1974; Ranatunga, 1977) while another proposes that a decrease in temperature may decrease the rate of removal of activator (Close & Hoh, 1968). In rabbit ventricular and papillary muscle the negative temperature coefficient is thought to be related to an alteration in excitation-contraction coupling involving an increase in the amount of Ca²⁺ released into the myoplasm in response to the action potential (Kaufmann & Fleckenstein, 1965; Langer & Brady, 1968).

To date, the tension-temperature relationship in the ABRM has not been studied in detail. However, using the method of Ritchie (1954) experiments performed in this study have shown that at 2 °C the duration of the active state is twice as long as at 20 °C (unpublished observations). This is similar to the case in frog skeletal muscle (MacPherson & Wilkie, 1954), and suggests that more Ca²⁺ may be released, or that its re-uptake may be slowed at the lower temperature (Close & Hoh, 1968).

Evidence for the latter is provided by the observations of Gogjian & Bloomquist (1977), who showed that Ca²⁺ uptake by vesicular organelles, isolated from the ABRM, was temperature-sensitive. However, the rate of uptake was reduced threefold

As the temperature was decreased from 30 to 10 °C while in this study tonic relaxation was reduced 13-fold over the same temperature range. A discrepancy, between rate of Ca²⁺ uptake by SR elements and relaxation, has been reported in other preparations; the reason is uncertain but it is suggested that Ca²⁺ may be taken up by a site which differs from its release site (Fuchs, 1974). The other possibility is that the rate of Ca²⁺ uptake by the SR *in vivo* is greater than *in vitro*.

Non-cycling cross bridges have been suggested to play a role in invertebrate catch muscle function (Twarog, 1976) and could be implicated in the effect of temperature on the ABRM. In particular, the observed increase in both the rates of contraction and relaxation with temperature may be explained in terms of non-cycling cross bridges, if these linkages retard both tension development and decay (Siegman *et al.* 1976; Dillon *et al.* 1981) and if their number is reduced as the ambient temperature increases. For the present, however, this hypothesis must remain a source of speculation and further investigation.

The observation that at temperatures greater than 27 ± 3 °C tonic relaxation becomes spontaneous (i.e. the application of relaxant is unnecessary) has been reported previously (Johnson, 1966; Twarog, 1967*b*; Bloomquist & Curtis, 1975). The reason for the disappearance of tension at this temperature has not been determined, but Johnson (1966) proposed that it may reflect a phase change in the paramyosin system. Twarog (1967*b*) offers two hypothesis; one, that temperature may directly affect the kinetics of an activator substance or, that the temperature effect is mediated by 5-HT which may be released from nerve terminals at a critical temperature. The mechanism of this effect has not yet been determined. However, in this study it was found that at, and above 30 °C, the half-times for 5-HT-induced and spontaneous relaxation approached one another, suggesting that above 30 °C relaxation is associated with, or mediated by, 5-HT release, or that the requirement for relaxant is lost.

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