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OSCILLATING CONTRACTIONS IN PROTOPLASMIC STRANDS OF *PHYSARUM*

MECHANICAL AND THERMAL METHODS OF PHASE SHIFTING FOR STUDYING THE NATURE OF THE SYNCHRONIZING FACTOR AND ITS TRANSMISSION*

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SUMMARY

A new experimental investigation chamber was used to analyse the control of rhythmic contractile activity in *Physarum*. A strand was mounted in such a way that isometric tension measurements of contraction forces could be made on two regions independently, the two regions remaining connected. It was possible to disturb one region experimentally and to compare its behaviour with the other.

A short time after being set up in the apparatus, the isometric contraction cycles in the two regions became synchronous. Stretching one region by 50% of its original length induced a phase delay relative to the other. A brief unilateral cold shock (Δt = 5–15 °C) had a similar phase-retarding effect. Synchrony was subsequently reattained, unless the connecting region was cut or, for example, treated with 30 mM benzamide.

In approximately 25% of the investigated strands, a rapid change to a higher temperature (Δt = 2–5 °C) caused the warmed side to be phase-advanced. However, 75% of the strands did not show a phase shift, suggesting that a rapid phase regulation is supported by increased temperature.

The described experimental assay is suitable for analysing the pathway and the nature of signal transmission in plasmodial strands.

INTRODUCTION

The plasmodia of the acellular slime mould *Physarum polycephalum* have been widely used as a model system to study the contraction physiology of cytoplasmic actomyosin (Fleischer & Wohlfarth-Bottermann, 1975). The longitudinal and radial contraction activities of the plasmodial veins show identical time periods and phases (Hülsmann & Wohlfarth-Bottermann, 1978), and one plasmodium seems to 'behave as a more or less synchronous system' (Grebecki & Cieslawska, 1978). Takeuchi & Yoneda (1977), Krüger & Wohlfarth-Bottermann (1978) and Yoshimoto & Kamiya (1978*a*) demonstrated that an unknown factor is responsible for the phase synchronization of the sinusoidal oscillating contraction activity.

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Since in the plasmodia, as in other non-muscle cells with autonomous locomotory activity, a nervous system capable of signal transmission is lacking, we focused our interest on the transmission of the regulating factor as an example of signal transmission in 'undifferentiated cells'.

It is not known whether a mechanical, an electrical or a chemical coupling is involved in the phase coordination and its spatial transmission. Yoshimoto & Kamiya (1978*c*) concluded that the flowing endoplasm 'must carry a factor(s) which coordinates the period and phase'.

The basis and stimulation of the present work was the elegant experimental procedure described by Takeuchi & Yoneda (1977): these authors demonstrated the spontaneous synchronizing capability of *Physarum* after a fusion of three different strands. Using another experimental assay, that is stretching procedures, Krüger & Wohlfarth-Bottermann (1978) independently arrived at the same conclusions. However, neither the fusion of different strands (i.e. *synchronization* as a criterion) nor stretching (i.e. dissynchronization and the following *resynchronization*) are satisfactory methods for studying signal transmission:

(i) Synchronization by fusion as a criterion depends upon estimating the time at which fusion is complete: this time is not exactly predictable and depends on many factors which cannot be governed by the experimenter.

(ii) In stretch procedures, the vein must be stretched by 50% (Krüger & Wohlfarth-Bottermann, 1978), which tears many of the strands.

Thus fusion is not time-reliable, and stretching is too time-consuming, because many of the strands tear during this procedure. The arrangement used in the stretching method also takes a considerable time to set up.

These shortcomings stimulated us to develop a more reliable and economical method for analysing the nature of the transmitted signal, i.e. a method allowing both unilateral phase shifts and experimental access to a connecting part of a strand trapeze, the pathway along which the synchronizing signal must be transmitted.

METHODS

Physarum polycephalum was cultured as described previously (Fleischer & Wohlfarth-Bottermann, 1975). Protoplasmic veins excised from plasmodia migrating on non-nutrient agar were mounted in twin tension transducers (Wohlfarth-Bottermann, 1977). The special investigation chambers are described in Results. Contraction forces were measured under isometric conditions. Temperature was controlled with the aid of a home-made Peltier element (see Wohlfarth-Bottermann, 1977), or (in pilot experiments) by rapid liquid-nitrogen cooling of the investigation chamber. The actual temperature of the investigation chambers was recorded electronically.

The results of this study are based on the tensiometric measurement of a total of 200 protoplasmic strands.

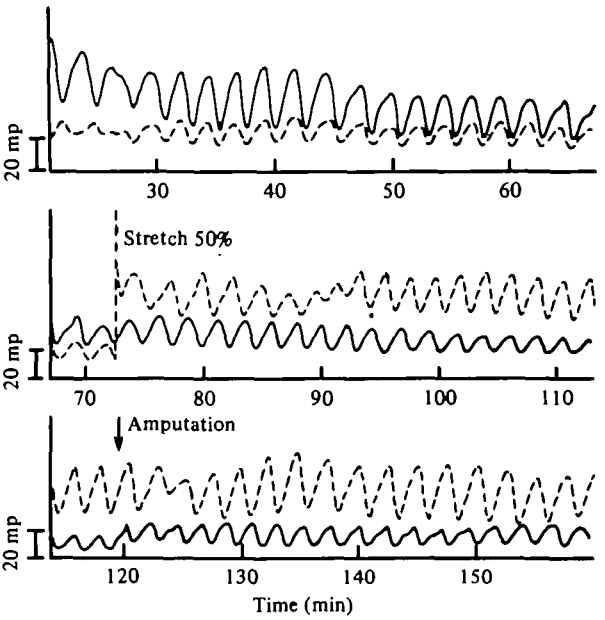

Fig. 1. Phase shifting (and resynchronization of phases) after stretching unilaterally one part (dotted curve) of a plasmodial strand mounted as a normal trapeze in a twin tension transducer. Note the phase shift (78 min) as the result of the stretch procedure (72·5 min), and the resynchronization of phases beginning at 100 min. The amputation (118 min) of the connecting part of the trapeze leads again to a (permanent) phase shifting. Compare with Fig. 6C.

RESULTS

Unilateral phase shifting by stretching and resynchronization of phases

Fig. 1 demonstrates the isometric contraction curves of simultaneous, independent measurements of both ends of one protoplasmic strand mounted in the form of a normal trapeze (Krüger & Wohlfarth-Bottermann, 1978). This arrangement (Fig. 3) allows one side of the strand to be stretched without mechanically affecting the other end and the connecting part of the vein. At 72·5 min, the strand with a total length of 22 mm was stretched unilaterally (Fig. 1, dotted curve) by a rapid elevation of the corresponding tensiometer head (see Fig. 3). A part of the strand that was originally 6 mm long was thus stretched to a length of 9 mm, a stretch of 50%. Between 78 and 96 min of the phase curves (Fig. 1), the stretching procedure resulted in a unilateral phase shift. This phase shifting between the two measured parts of the strand disappears at 100 min. Obviously, the strand is able to resynchronize the contraction activities of both vein parts in spite of the fact that now both parts are under different isometric tension. At 118 min, the connecting part of the strand was interrupted mechanically by amputation. This leads to a renewed and permanent phase shifting, obviously because the connecting strand, i.e. the carrier transmitting the regulation signal, is now interrupted. Fig. 6A–C summarizes diagrammatically the phase behaviour as a reaction to stretch stimuli applied unilaterally to protoplasmic strands in a trapeze arrangement.

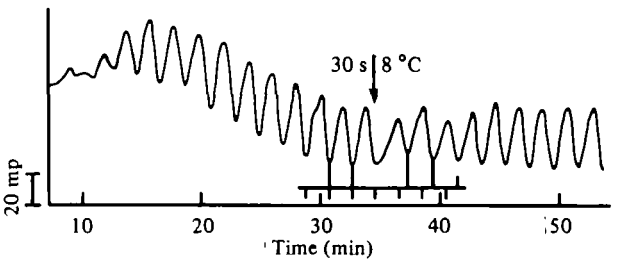

Fig. 2. Experimental phase shifting by temporary low temperature (22→8 °C for 30 s). Phase shifting is demonstrated by extrapolation (indicated on the abscissa) of the minima (before the temperature experiment was begun) to the time after the temperature experiment. Compare with Fig. 6 D_1, D_2.

Unilateral phase shifting by temperature changes and resynchronization of phases

Fig. 2 represents the isometric contraction curve of a longitudinally mounted, single strand. During the time interval between 34·5 and 35 min, i.e. for a total time period of 30 s, the temperature of the strand (working at room temperature 22 °C) was quickly lowered to a temperature of approximately 8 °C by moving a small tank containing liquid nitrogen close to the strand. After this 30 s cooling period, the strand was allowed to rewarm to room temperature. Temperature changes (Δt = 10–15 °C) induced in this way in the vicinity of the strand were measured electronically. This sudden decrease in temperature down to 8 °C leads to a temporary suppression of force output of the oscillating contraction activity, i.e. in a decrease of frequency (Fig. 2). The frequency increases again as soon as the strand warms up to room temperature. The occurrence of a phase shift can be demonstrated by extrapolating the original minima or maxima of the curve (before the cooling) to the time course of oscillation after rewarming. As shown by Krüger & Wohlfarth-Bottermann (1978), the extrapolation is reliable for an evaluation of the occurrence of a phase shift for a limited time period of approximately 15 min only.

Experiments with single veins, and a rapid short-time cooling device as described above, revealed that sudden temperature changes (Δt = 10–15 °C) of the strands represent a reliable method for the experimental induction of any wanted phase shift. Moreover, it was clear that such experiments were more easily performed as stretch procedures, especially when a trapeze arrangement is indispensable as a reference, i.e. a control curve resulting from the experimentally unaffected part of the strand. This control pattern is needed, because the extrapolation method is limited in its reliability to a time range of 15 min.

Simultaneously, a trapeze arrangement with its connecting strand transmitting the synchronization signal offers an elegant experimental arrangement for testing mechanical, chemical and other influences which might inhibit the signal transmission along this pathway. Experimental influences along the connecting strand might thus deliver information concerning the unknown nature of the signal.

For a routine assay, we constructed a double chamber (Fig. 3) fullfilling the following conditions:

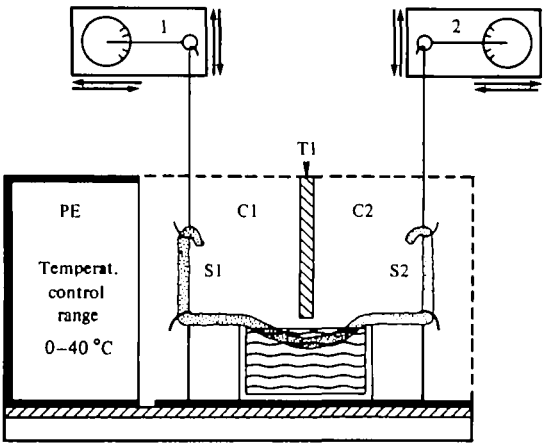

Fig. 3. Diagram of the experimental arrangement for investigating thermal-induced phase shifts of one part of a strand mounted as a normal trapeze in a twin tension transducer. 1 and 2: Tension transducers, adjustable in horizontal and vertical directions (arrows). PE: Peltier element for rapid unilateral temperature control of the left portion of the strand in chamber C1. T1: Thermal isolation separating chambers C1 and C2. The plate for thermal isolation is arranged as a movable door. S1 and S2: Different parts of one continuous strand. The longitudinal contraction activities of both lateral parts of the strand are registered simultaneously under isometric conditions of measurement. The horizontal part of the strand connecting S1 and S2 runs through a small pot to which test solutions can be added. - - -, Moist chamber with a volume of approx. 70 cm^3. Total length of the vein 3 cm.

(1) Unilateral rapid temperature control (PE) in one of the chambers (C1) without affecting considerably the constant room temperature in the other chamber (C2).

(2) Simultaneous isometric measurements (1, 2) of both strand ends (S1 and S2) of one vein (trapeze) in different chambers.

(3) Experimental accessibility of the connecting horizontal part of the vein for a spatially (6 mm) limited application of mechanical and chemical influences during continuous measurement of the phase behaviour of the two parts of the strand in different chambers.

(4) Prevention of air drying of the strands during long term experiments by securing a permanent humid atmosphere around the object.

Fig. 3 is a diagram of this investigation chamber which proved to be efficient for performing experimental series with phase shifting by increased as well as by decreased temperatures. Since the preparation of a trapeze arrangement and the registration of its behaviour is a relatively time consuming procedure, the mechanical construction of this double chamber should allow rapid and precise manipulations during the course of the experiment.

The reaction of different strand parts to unilateral temperature *decrease* in the range of $\Delta t = 5$ °C is demonstrated in Fig. 4. The phase curves of this strand are shown from the beginning, i.e. immediately after its isolation from the substrate and the construction as a trapeze. It takes about 10 min for both strand ends to assume the regular pattern of oscillation. A further 30 min is needed before phase synchronization of both ends of the vein is accomplished (time point 40 min in Fig. 4). We registered

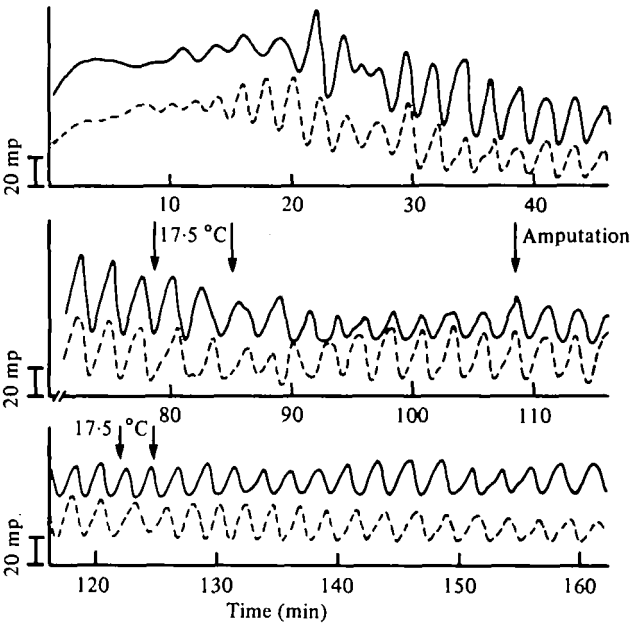

Fig. 4. Reaction of a plasmodial strand under experimental conditions as described in Fig. 3. A temporal unilateral lowering of temperature (79 min to 85 min) from 22 °C to 17·5 °C results in a phase shift. The strand performs a resynchronization within a time period of 12 min. After amputation of the connecting part (108 min) a second thermal phase shift (122–125 min) resulted in a permanent phase shifting.

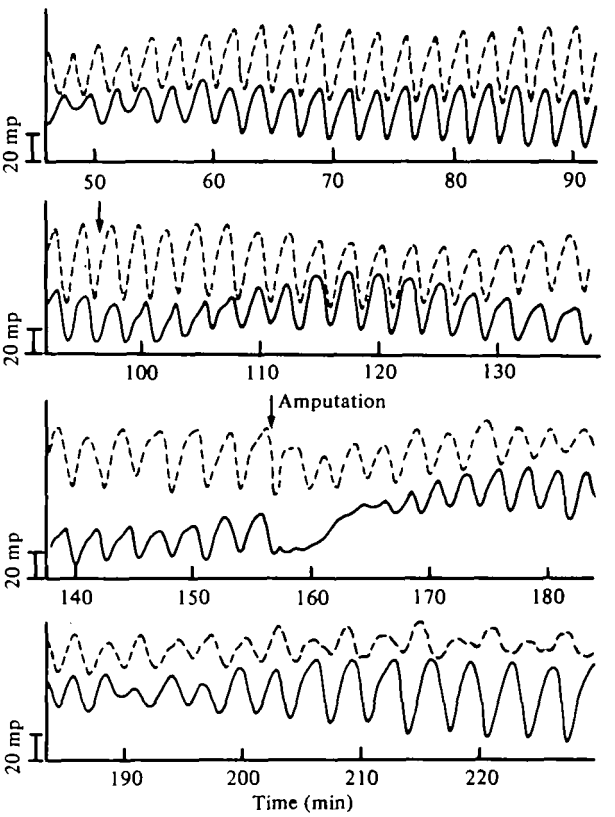

Fig. 5. Reaction of a trapeze upon a permanent temperature gradient of 2 °C beginning at 97 min (first arrow). Note the phase shift between 100 and 105 min and the following resynchronization. Amputation of the connecting strand at 157 min results in a permanent phase shifting (Fig. 6E_3, E_4).

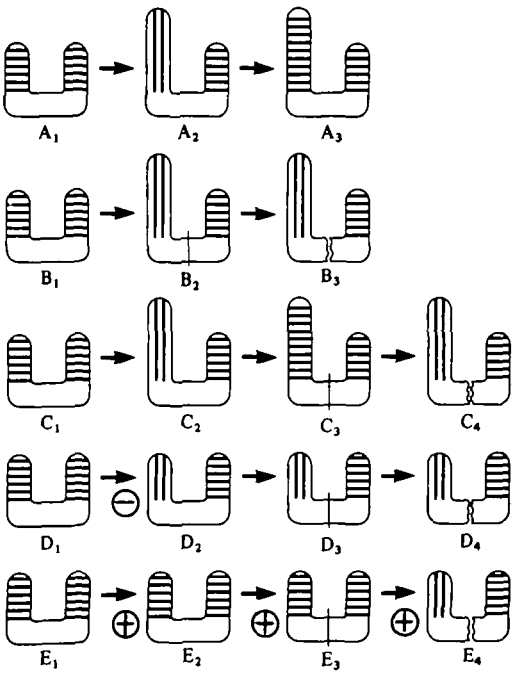

Fig. 6. Diagrammatic representation of the results. (A) Phase shifting by unilateral 50% stretch (2), and spontaneous resynchronization (3). (B) Phase shifting by unilateral 50% stretch (2), and persistence of phase shift after amputation (3). (C) Resynchronization of phases (3) after a 50% stretch-induced phase shift, and renewed spontaneous phase shifting after amputation (4). (D) Phase shifting by a short unilateral temperature decrease (3) and its persistence after amputation (4). Δt = 5–15 °C. (E) Maintenance of phase synchronization in spite of a permanent unilateral temperature increase (2, 3), and phase shifting after amputation (4). Δt = 2–5 °C.

the maintenance of this synchronicity for a time period of 40 min in order to be sure that no spontaneous phase changes occurred. After this routine 'equilibration period', a unilateral temperature change resulted in phase shifts between the two strand ends (time point of 78 min in Fig. 4). In this case, the temperature within the controlled chamber was lowered from room temperature (22 °C) to approximately 17·5 °C for 6 min. Immediately after this cooling procedure, the controlled chamber was rapidly heated to 22 °C. The curves in Fig. 4 demonstrate that the thermal-induced phase shift by cooling disappears at 98 min, i.e. there is a complete phase synchronization of both parts of the strand.

Another thermal phase shift was induced at 122 min, after the connecting part of the strand had been amputated at 108 min. Under these conditions, however, the oscillating contraction activity of both strand parts is not resynchronized (see time span 125–160 min). This result shows that the connecting part of the strand is responsible for the transmission of the synchronizing signal. This carrier can be used to test the effects of different substances on its function, i.e. on signal transmission (see Fig. 6D). Pilot experiments with different drugs showed the convenience of the described assay for a blockage of signal transmission (Fig. 7).

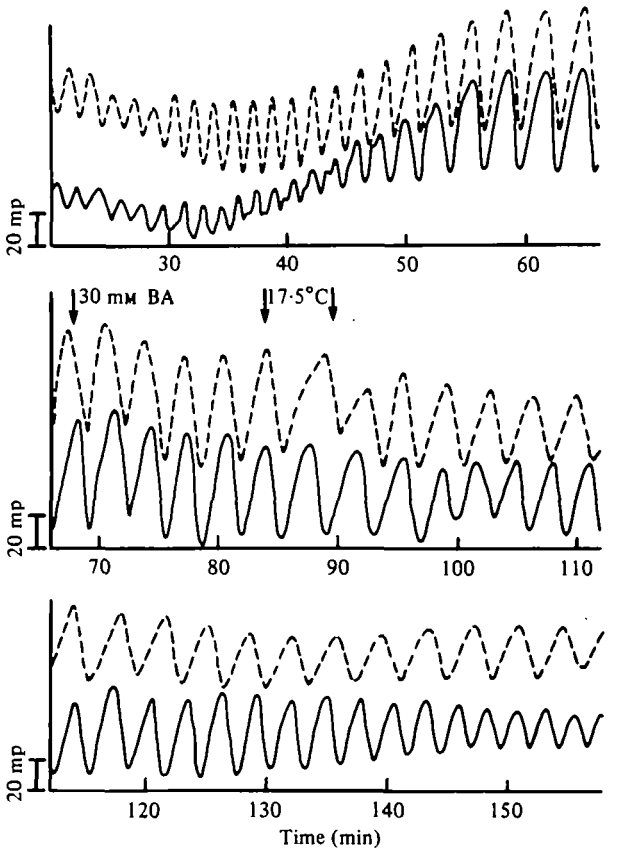

Fig. 7. The external application of 30 mM benzamide (BA) to the connecting strand part (69 min) does not lead to an alteration of the synchronous behaviour. The following temperature-induced phase shift (83 min) shows that the vein has lost the ability for phase coordination.

In another series of experiments, we tested the use of a unilateral temperature *increase* (Δt = 2–5 °C) for experimental phase shifting. In contrast to a lowering of temperature, however, it showed that temperature increase often does *not* lead to phase shifts unless the connecting piece of the strand is interrupted by amputation.

Fig. 5 demonstrates an experiment in which a phase shift was produced at 97 min by a temperature difference of 2 °C. This temperature gradient was maintained permanently, i.e. from 97 min onward to the end of the experiment. As a reaction of the unilateral warming, there is a phase shift from 100 to 108 min followed by a re-synchronization of phases. The interruption of the connecting strand piece at 156 min results in a loss of synchronization capability (Fig. 6E).

The diminished amplitudes of force output of the strand part working at 24 °C (Fig. 5, 200–230 min, dotted curve) in comparison to the control pattern (22 °C) often appear in a trapeze arrangement within a permanent thermal gradient. This diminished force output may be interpreted as a thermotactic reaction of the object.

In many cases a permanent temperature gradient of 2 to 3 °C in the vicinity of room temperature does *not* lead to a phase shift, i.e. the veins are able to overcome this

gradient by regulation, unless signal transmission along the connecting part becomes inhibited (Fig. 6E). Therefore, the stability of synchronization within a permanent temperature gradient is a reliable criterion for an intact pathway of the synchronizing factor.

In Fig. 6 the present results are summarized diagrammatically as a basis for the following discussion, and to show which of the described methods should be used as a routine assay to analyse the nature of the transmitted synchronization signal.

Fig. 7 is intended to show that the interruption of signal transmission along the connecting strand can be performed not only physically, e.g. by amputation, but also by an external treatment of the intact connecting piece with chemical substances (Fig. 3). A solution of 30 mM benzamide was used, which is known to have a strong relaxing effect when applied externally (Korohoda & Wohlfarth-Bottermann, 1976).

In the experiment shown in Fig. 7, the test solution was added to the connecting part of a synchronously working trapeze at 68 min. Between 70 and 80 min the test solution does not seem to have a recognizable effect upon force output or synchronous behaviour. The following phase shifting by a unilateral, short temperature decrease (85 min) reveals that the trapeze has lost its capability of phase synchronization, obviously only due to the effect of the externally applied benzamide. Control experiments with distilled water and ionic solutions revealed an unaltered synchronous behaviour.

DISCUSSION

Phase shifting by stretch

The ability to perform phase shifts by mechanically stretching the strands is of general interest for investigating whether mechanical tension forces are involved in the enigmatic regulation mechanism governing cyclic contraction activity.

Krüger & Wohlfarth-Bottermann (1978) described phase shifts of the contraction–relaxation cycles induced by stretching the strands by one half their original length (50%). Yoshimoto & Kamiya (1978*b*), however, could not reveal phase shifts by stretching, and therefore deny that tension forces are involved in the regulation mechanism. Independent of the question whether mechanical tension forces play a role in the normal cycle control, the results of our study (Fig. 1, Fig. 6A–C) confirm previous results (Krüger & Wohlfarth-Bottermann, 1978) according to which phase shifting by strong stretch procedures represents a reproducible response of the strands. Therefore, we conclude that tension forces should not be excluded in the discussion of factors possibly involved in cycle regulation.

Furthermore, stretch-induced unilateral phase shifts of strands in a trapeze arrangement in principle can be used to study the signal transmission mechanism. This is valid for a construction of the strand as an inverse trapeze (Krüger & Wohlfarth-Bottermann, 1978) as well as for a strand in the form of a normal trapeze (this study, see Fig. 3). The normal trapeze arrangement has two advantages: (1) construction is more easily performed, and (2) a transmission of forces resulting from the connecting, horizontal part of the strand to the measuring devices is excluded, because this part of the strand extends between two immovable fixed points (tensiometer lift).

For experiments with a high number of strands, i.e. for a routine test assay,

unilateral stretch procedures in a trapeze arrangement (Fig. 6B, C) are a rather time consuming method, since not all veins withstand the stretch procedure. Therefore we prefer a phase shift by temperature as the routine test method.

Phase shifting by temperature

Obviously, the 'thermal method' of phase shifting for studying the resynchronization mechanism is more reliable and easier to perform routinely than the stretching procedure. Preconditions for such experiments are well constructed investigation chambers allowing simultaneously a rapid unilateral temperature control, a convenient experimental accessibility of the object, and the preservation of air humidity in the interior of the chambers (Fig. 3).

As was shown in Figs. 2, 4, 5 and 7, the physiological clock of the oscillator is temperature-dependent (see Wohlfarth-Bottermann, 1977), and thus the temperature can be used to shift the timing of the clock, i.e. to shift the phase of the oscillating cycle (Fig. 6D, E). In fact, the clock can be arrested by a rapid temperature decrease down to 2–5 °C. In comparison to a mechanical stretch shifting, the thermal phase shift within physiological temperature limits has the advantage of being a rapid, and *completely reversible* influence upon the spontaneous contraction behaviour of the object. The available temperature range for changing the room temperature (22 °C) is approximately 8–28 °C.

It is remarkable that upon unilateral temperature decrease a rapid phase shift can be observed, whereas a temperature increase with identical Δt is compensated for, in the sense that a phase shifting frequently does not occur. This means that the ability to regulate is supported by a temperature increase until the upper physiological limit of approximately 28 °C is reached.

General considerations

Obviously, an unknown synchronizing factor exerts its effect, resulting in a self-perpetuating system of synchronized contraction events along a protoplasmic strand of several centimeters in length. This paper was devoted to the development of a convenient routine method to investigate the nature of this contraction synchronizing factor, which is transmitted along a protoplasmic strand, and obviously responsible for the synchronization of contraction rhythms (Takeuchi & Yoneda, 1977; Grebecki & Cieslawska, 1978; Krüger & Wohlfarth-Bottermann, 1978; Wohlfarth-Bottermann, 1979; Yoshimoto & Kamiya, 1978*b*).

The forecast of contraction phases based on the previously registered contraction rhythmicity (extrapolation method, see Krüger & Wohlfarth-Bottermann, 1978) is limited to a time span of approximately 15 min because of the fact that spontaneous changes of frequency, i.e. phase shifts in one measured vein, occur relatively frequently. Therefore, extrapolation methods do not represent a reliable way to study phase shifting and its regulation over longer periods of time.

The trapeze method has the advantage not only of delivering a control curve in the form of the second (apparently unaffected) portion of the strand but also of offering a connecting part of the bilaterally measured strand where an experimental access of the carrier for testing the nature of the transmitted signal is available.

Measuring the force output of both strand parts simultaneously after a unilateral

experimental influence, one curve can be looked upon as a test pattern and the other as the control pattern of the oscillating activities. However, in the case of a functional information system between both vein parts, it must be taken into consideration that the control pattern may also be influenced by regulatory effects issued from the experimentally affected strand part.

According to our current experience, a short temperature decrease (Fig. 6D) as well as a permanent temperature increase (Fig. 6E) can be used. In practice, we have started a series of investigations testing different substances with both methods simultaneously (Fig. 6D and E) using two twin tension transducers.

According to Fig. 6D and E two different criteria can be used for evaluating an unaffected or a blocked synchronizing capability of the connecting strand piece:

(1) the ability of the trapeze to *resynchronize* a phase shift by a short temperature decrease (Fig. 6D), or

(2) the *maintenance* of synchronicity in spite of a permanent unilateral temperature increase (Fig. 6E).

Results concerned with the application of both methods will be given in a following paper. In connexion with appropriate experimental intervention, we anticipate that phase response curves resulting from phase-shifted trapezes will deliver information concerning nature and pathway of the signal, its transmission along the longitudinal axis of the strands and its transduction to a phase coordination. Such an increased knowledge of the regulatory mechanism may additionally contribute information about the nature of the oscillator.

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