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Source: Zoological Science, 12(4) : 397-403

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.12.397

ZOOLOGICAL SCIENCE 12: 397–403 (1995)

Phase-Response Analysis of Stretch-Mediated Beat Coordination in the Oyster Heart. I. Phase-Response Characteristics of Auricle and Ventricle to Brief Stretches

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ABSTRACT—The mechanisms of beat coordination in the heart of the oyster *Crassostrea gigas* were analyzed by examining phase-response characteristics of both the auricle and ventricle to brief stretches. Brief stretching of an isolated auricle or ventricle produced phase delay or phase advance in the following beats, in accordance with the stimulation phase. Therefore, the phase-response curve (PRC) was always biphasic and the phase shift was larger with a larger stretch. As predicted from the PRC, the beat frequency of an isolated auricle or ventricle was entrained by repeated brief stretches when the stretch frequency was in a limited frequency range (range of entrainment) around the free-running beat frequency. The range of entrainment was wider with a larger stretch. When the stretch frequency was outside the range, the beat frequency was not entrained. Instead, it changed cyclically as a process of repeated discrete phase shifts. These results suggest that the beat coordination of the auricle and ventricle is achieved by reciprocal stretching between them.

INTRODUCTION

The molluscan heart is situated in the pericardial cavity with its venous and aortic ends attached to a tough pericardial wall. When the heart is isolated and kept unstretched, the auricle and ventricle beat independently with their own rhythms [6, 9]. However, when the isolated heart is stretched, the auricle and ventricle beat alternately. Willems [17] demonstrated in a snail heart that when the auricle and ventricle were isolated separately, they beat with their own respective rythms, but alternate beating was restored when they were connected with a silver wire and kept stretched. These facts strongly support the idea that reciprocal stretching between auricle and ventricle plays an important role in the establishment of a coordinated beat in the molluscan heart. The details of the mechanisms behind the stretch-mediated coordination have not yet been investigated.

The molluscan heart generally responds to continuous stretching by increasing the rate of rise of the pacemaker potential and results in increase in the beat frequency [1, 10, 11]. However, a brief stretch applied to an isolated auricle or ventricle of the oyster produced different effects on the periodic membrane potential change of the myocardium and prolonged or shortened the beat period depending on the timing of application [15, 16].

In this paper, the phase-response characteristics to brief stretches are presented for auricle and ventricle of the oyster. We also examine the entrainment of the beat rhythm of the auricle and ventricle by repeated brief stretches. The aim is to understand the mechanisms of stretch-mediated beat coordination in the oyster heart.

Accepted May 29, 1995
Received March 20, 1995

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MATERIALS AND METHODS

Preparations and electrical recordings

Specimens of the Japanese oyster (*Crassostrea gigas*) weighing between 50 to 150 g were collected at the shore of Nakaminato and Shimoda, Japan. They were kept in a laboratory aquarium for a maximum of three weeks at 15 to 18°C before experimentation. The upper (right) valve of an oyster was removed and the heart exposed by removing the upper wall of its pericardial cavity. The auricle or ventricle was then removed from the cavity. One end of the isolated auricle or ventricle was pinned to the bottom of an experimental chamber and the other end was connected to the tip of a galvanometer for a pen-writing oscillograph to stretch the tissue [16]. The galvanometer was driven by a electric stimulator and controlled by a feedback amplifier. The tissue was stretched for 50 to 500 msec by an extent to 0.4 to 1.3 mm (5 to 25% of its total length). The stretch was monitored by an electric signal from a mechano-electric transducer attached to the same end of the tissue as the stimulator. The action potential was recorded using a glass suction electrode (inner diameter, 100 to 150 μm) placed at the pinned end of the preparation. The experimental chamber was perfused with aerated artificial sea water (ASW) of the following composition (in mmol/l): NaCl 462, KCl 9, $CaCl_2$ 9, $MgCl_2$ 36, $MgSO_4$ 17, Tris-HCl buffer (pH. 7.2–7.4) 6.

All electrical signals (action potentials and signals monitoring the stretch) were amplified, and displayed on a cathode ray tube, and recorded on magnetic tape and/or a pen recorder chart. All the experiments were performed at a temperature of 20 to 24°C.

Determination of the phase-response curve

Since an auricular or ventricular beat was always accompanied by an action potential [15], the beat interval was defined as the time from the peak of one action potential to the next. When deviation around the mean interbeat interval of eleven successive action potentials, under non-stimulated (free-running) conditions, was less than 1%, the preparation was used for examining the effect of stretch on the beat. A brief stretch was applied to the tissue to obtain a phase-response curve (PRC) (e.g. [12–14]). The stretch (stimulation) phase was defined as the period of time from the peak of the action potential preceding the stretch to the onset of the stretch, and indicated by percent of the mean interbeat interval (control beat interval). The phase shift was defined as the time shift of the peak of the action potential after the stretch, and presented as a percent of the control beat interval. During experimentation, the shift of the peak in the second or third action potential after the stretch was determined. In a single preparation, 50 to 200 stretches were applied at various phases, in random order. Control of the stretch phase and determination of the phase shift were performed with the aid of microcomputer system. The phase shift was displayed on a cathode ray tube as a function of the stretch phase to obtain the PRC.

RESULTS

Phase-response curve of auricle and ventricle to brief stretches

A brief (50 to 500 msec) stretch was applied to an isolated auricle or ventricle at various beat phase, and effects on the beat period were examined. As shown in Figure 1 (A; auricle, B; ventricle), when a stretch was applied shortly after the peak of the action potential (A1 and B1), the next beat was delayed. In contrast, when a stretch was applied at the later phase of the action potential (A2 and B2), the next beat was advanced. These results are consistent with our earlier works [15, 16]. A brief stretch, therefore, can be said to cause a phase shift in subsequent beat cycles.

In order to visualize the relationship between the moment of stretch and the phase shift, PRC's were drawn as shown in Figure 2 (A; auricle, B; ventricle). Each curve was biphasic, showing phase delay (positive phase shift) and phase advance (negative phase shift) as a function of the phase at which the stretch was applied (stimulation phase). The phase shift changed from delay to advance at a stimulation phase of about 40% (30 to 50% depending on preparations). It changed from advance to delay again at about 90%. No difference in the PRC's was found between the

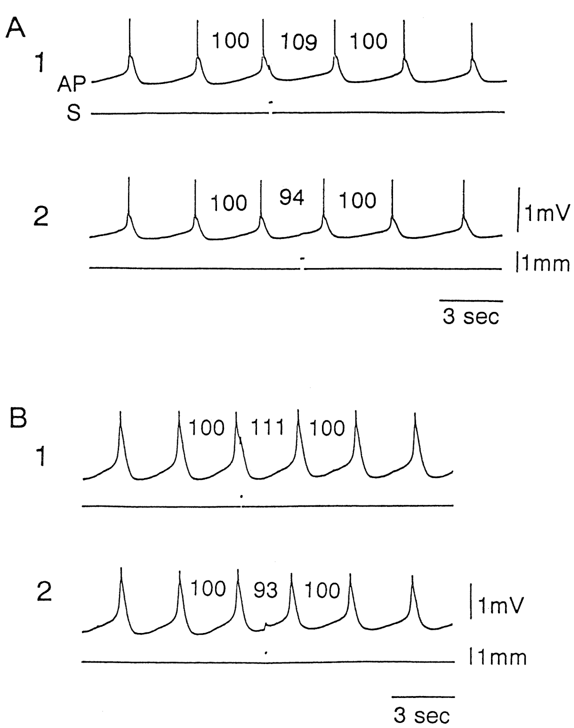

FIG. 1. Effects of a brief stretch on the beat rhythm of an isolated auricle (A) and of an isolated ventricle (B). A stretch (duration; 200 msec, magnitude; 0.5 mm) was applied in early beat phase (10%) in A1 and in late beat phase (61%) in A2. Similarly a stretch (duration; 100 msec, magnitude; 0.5 mm) was applied in early beat phase (9%) in B1 and in late beat phase (50%) in B2. AP; action potential recorded extracellularly. S; monitored stretches applied. Changes in the beat interval are shown as a percent of the interval of free-running beat.

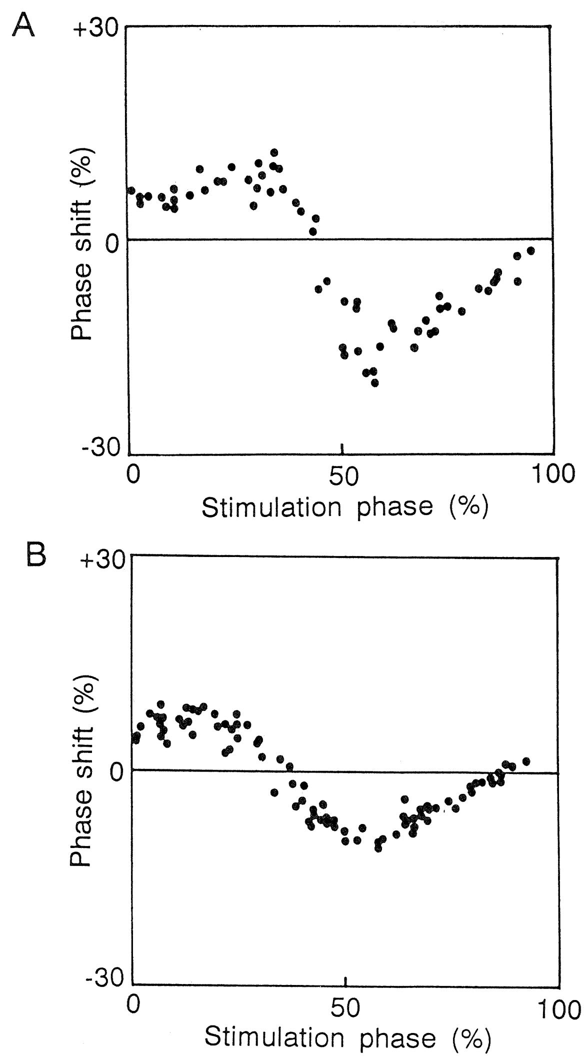

FIG. 2. Phase-response curves (PRC's) for brief stretches of an isolated auricle (A) and of an isolated ventricle (B). Duration of stretch; 50 msec in A, 100 msec in B. Magnitude of stretch; 0.6 mm in A, 0.8 mm in B. Free-running beat interval; 3.25 to 3.62 sec in A, 2.14 to 2.32 sec in B.

auricle and ventricle.

The effect of the magnitude of stretch on the PRC was examined. Two PRC's were drawn in the same auricle or ventricle preparation with two different magnitudes of stretch. As shown in Figure 3, the phase shift was larger with a larger stretch.

The effect of duration of stretch on the PRC was also examined. No definite difference in the PRC's was found between two different duration of stretch in the range used in this study.

Effects of repeated brief stretches on the beat rhythm

The PRC's obtained (Figs. 2, 3) suggest that the beat rhythm is entrained by repeated mechanical stretches [12–14]. Therefore, repeated brief stretches were applied to the isolated preparations. No noticeable difference in the effect was found between the auricle and ventricle. Figure 4 shows an example obtained from the isolated ventricle. When the frequency of stretch was about 6% lower (B) or about 10% higher (C) than the free-running beat frequency (17.6 beats/min), the beat rhythm was entrained to the stretch frequency. When the stretch frequency was about 13% lower (A) or about 17% higher (D), the beat rhythm was not entrained. Instead, the beat frequency changed cyclically. The beat rhythm was thus entrained to the stretch frequency within a limited frequency range (range of entrainment) around the free-running beat frequency.

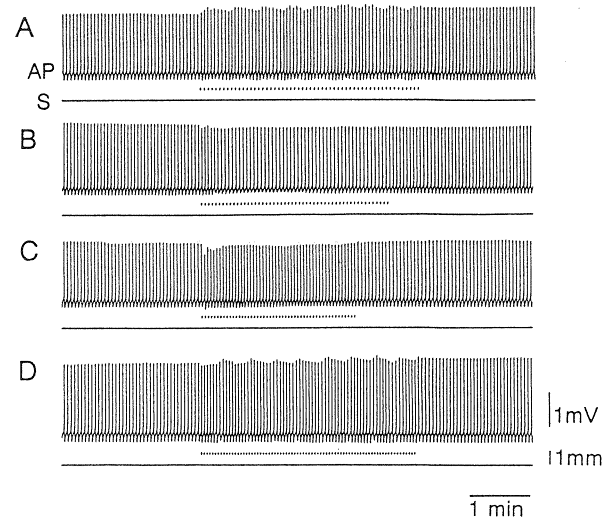

Fig. 4. Effects of repeated brief stretches on the beat rhythm of an isolated ventricle. Duration of stretch; 500 msec. Magnitude of stretch; 1.2 mm. Stretch interval; 3.90 sec in A, 3.60 sec in B, 3.10 sec in C, 2.90 sec in D. Free-running beat interval; 3,40 to 3,47 sec. AP; action potential recorded extracellularly. S; monitored stretch applied.

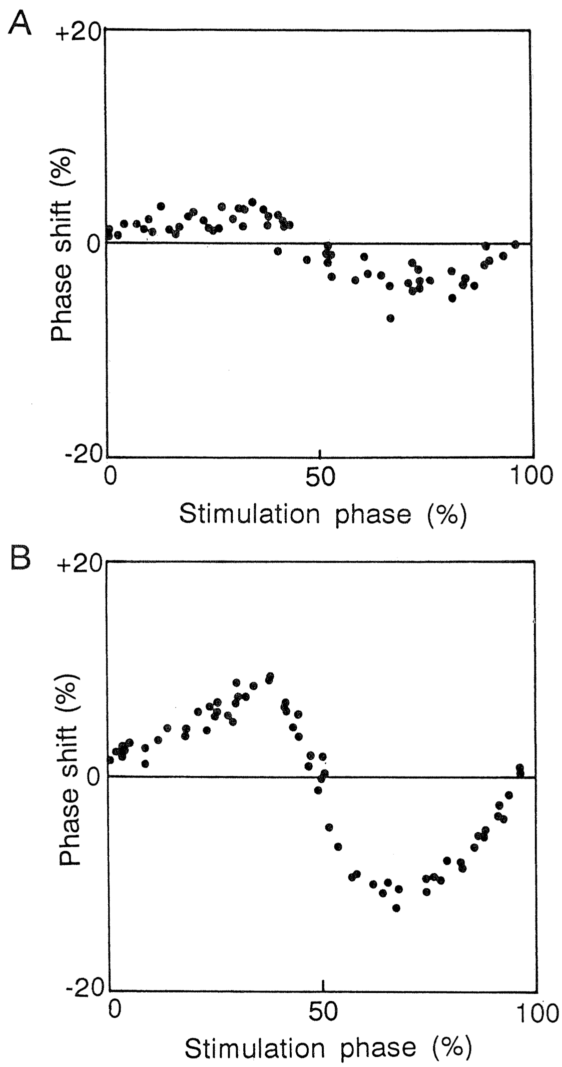

Fig. 3. Effects of magnitude of stretch on the PRC of an isolated auricle. Duration of stretch was kept constant at 50 msec, while magnitude of stretch was varied (A; 0.5 mm, B; 0.7 mm). Free-running beat interval; 2,45 to 2,62 sec.

To examine the relationship between the magnitude of stretch and the range of entrainment, two different magnitudes of repeated stretches were applied at the same frequency to the same isolated preparation. Figure 5 shows an example obtained from an isolated ventricle. To visualize the beat frequency change, each beat interval was determined before and during the repeated stretches and plotted against time (the same rule is applicable to the figures that follow). When the stretch frequency was about 7% lower or higher than the free-running beat frequency (16.9 beats/min), the beat frequency changed cyclically with the small (magnitude; 0.8 mm) stretches (A1, A2), whereas the beat frequency was entrained to the stretch frequency with the large (magnitude; 1.0 mm) stretches (B1, B2). The range of entrainment was thus wider with larger stretches.

Phase relation between brief stretches and the entrained beat

In the first series of experiments, we examined phase relation between each stretch and each beat after the beat was entrained by repeated brief stretches. Figure 6A shows examples obtained in an isolated ventricle in which the beat rhythm was entrained to six different stretch frequencies. When the stretch frequency was lower (a; 1.6, b; 4.6, c; 7.4%) than the free-running beat frequency (19 beats/min),

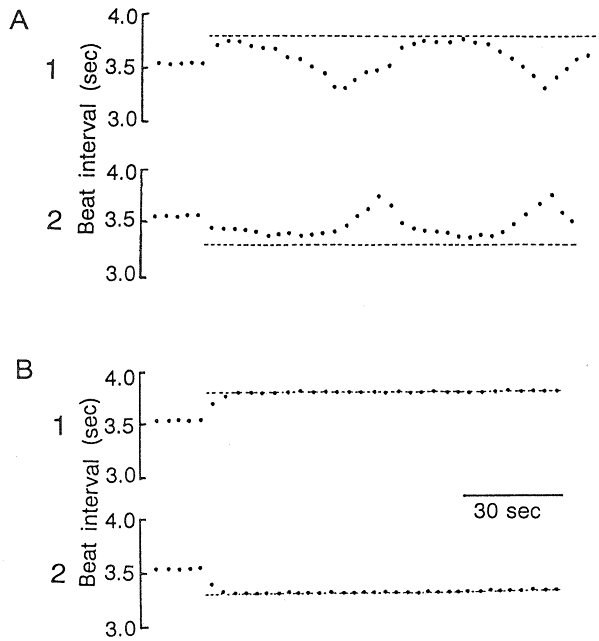

Fig. 5. Effects of magnitude of stretch on a range of entrainment in an isolated ventricle. Duration of stretch was kept constant at 500 msec, while magnitude of stretch was varied at 0.8 mm in A and 1.0 mm in B. Stretch interval; 3.80 sec in A1 and B1, 3.30 sec in A2 and B2. Free-running beat interval before and during repeated stretches is successively plotted against time. Dotted lines indicate intervals of the stretch applied.

location of each stretch was always in an early phase of each beat. When the stretch frequency was higher (d; 1.6, e; 5.0, f; 8.6%) than the free-running beat frequency, location of each stretch was always in a late phase of each beat. The precise relationship between the location of each stretch (stretch phase) and resultant change in the beat frequency is shown in Figure 6B. A change in the beat frequency is expressed as a percent change in the beat interval relative to the interval corresponding to the free-running beat frequency (the same rule is applicable to the figures that follow). In the figure, each change in ten successive entrained beats is plotted against each stretch phase. The relationship is consistent with the PRC.

In the next series of experiments, the time course of the entrainment was examined. The stretch frequency was lower (about 6%) than the free-running beat frequency (18.4 beats/min) in the cases shown in Figure 7. When the first stretch stretch was applied in early beat phase (A1), the beat interval first became longer than the free-running beat interval, gradually approaching the stretch interval (B; solid circles). By contrast, when the first stretch was applied in late beat phase (A2), the beat interval first became shorter than the free-running beat interval and then gradually approached the stretch interval (B; open circles). In both cases, the location of stretch (stretch phase) shifted progressively and was finally fixed in an early phase of the beat (A1, A2). The each change in the beat interval is plotted against the each stretch phase (C). The relationship is consistent with the PRC.

In the cases where the stretch frequency was higher than the free-running beat frequency, the stretch phase shifted progressively and was finally fixed in a late phase of the beat. The relationship between each change in the beat interval and each stretch phase was consistent with the PRC (data not shown).

Phase relation between repetitive stretches and the non-entrained beat with cyclically changing beat intervals

We examined phase relation between each stretch and each beat while the beat frequency changed cyclically. The

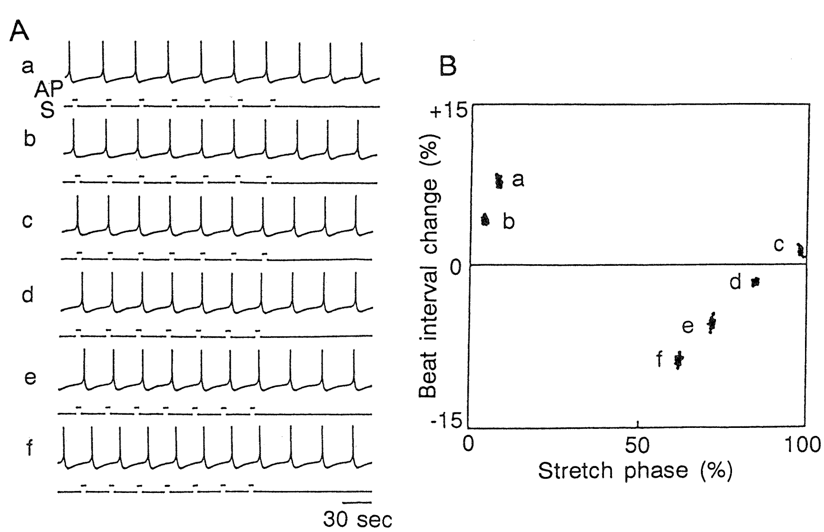

Fig. 6. Phase relationship between repeated brief stretches and entrained beats in an isolated ventricle. A: Action potentials recorded extracellularly (AP) and stretches applied (S). Duration of stretch; 500 msec. Magnitude of stretch; 1.0 mm. Stretch interval; 3.40 in a, 3.30 in b, 3.20 in c, 3.10 in d, 3.00 in e and 2.90 sec in f. Free-running beat interval; 3.14 to 3.16 sec. B: Relationship between stretch phase and the change in the beat interval. Changes in ten successive beat intervals after entrainment were plotted against each corresponding stretch phase. Each letter (a-f) corresponds to that in A.

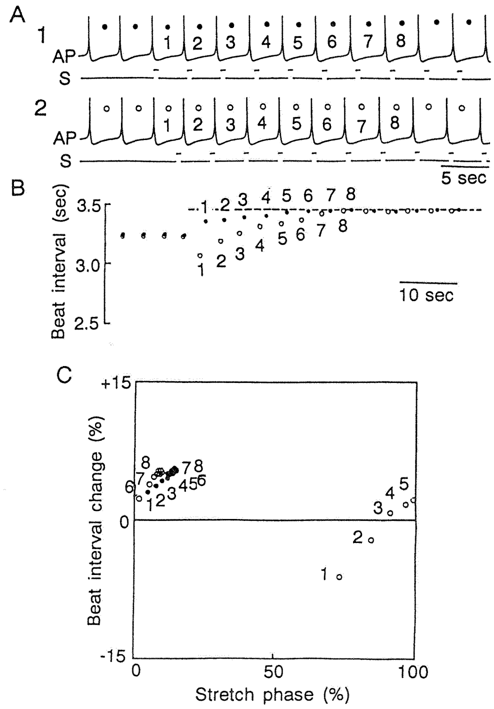

FIG. 7. Phase relationship between repeated brief stretches and beats during establishing entrained beats in an isolated ventricle. A: Action potentials recorded extracellularly (AP) and stretches applied (S). Duration of stretch; 500 msec. Magnitude of stretch; 1.0 mm. Stretch interval; 3.45 sec. The first stretch was applied in early beat phase (5%) in 1 and in late beat phase (73%) in 2. Free-running beat interval; 3.24 to 3.26 sec. B: Time course of change in the beat interval by repeated stretches. Dotted line indicates the stretch interval. Numbered plots correspond to numbered beat intervals in A (1; solid circles, 2; open circles). C: Relationship between the stretch phase and the change in the beat interval. Numbered plots correspond to those in A and B.

stretch frequency was lower (about 10%) than the free-running beat frequency (18.9 beats/min) in the case shown in Figure 8. When the first stretch was applied at an early phase in the beat cycle (A1), the beat interval increased successively towards the stretch interval and then changed cyclically (B). Since the beat interval was always shorter than the stretch interval (B), the location of stretch (stretch phase) shifted successively (A). The relationship between the each stretch phase and the each change in the beat interval is consistent with the PRC (C).

In the cases where the stretch frequency was higher than the free-running beat frequency, the stretch phase shifted successively and the beat interval changed cyclically. The relationship between the each stretch phase and the each change in the beat interval is consistent with the PRC (data not shown).

We next examined the relation between the cyclical change of beat frequency and the stretch frequency in isolated preparations. Figure 9 shows an example in which repeated brief stretches were applied at two lower (7 and 10%) and two higher (13 and 18%) different frequencies than the range of

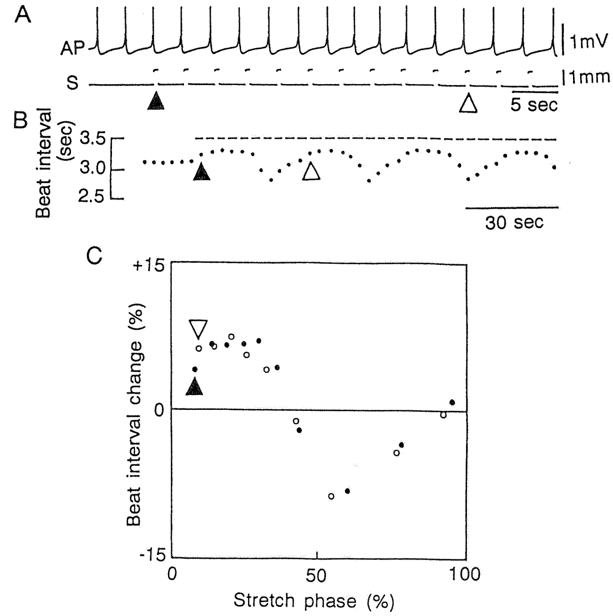

FIG. 8. Phase relationship between repeated brief stretches and non-entrained beats with cyclical changing beat interval in an isolated ventricle. A: Action potentials recorded extracellularly (A) and stretches applied (S). Duration of stretch; 500 msec. Magnitude of stretch; 0.8 mm. Stretch interval; 3.50 sec. Solid triangle indicates the first stretch (stretch phase; 8%). Open triangle indicates the 11th stretch, which was applied at a beat phase almost the same as the first stretch. Free-running beat interval; 3.12 to 3.18 sec. B: Time course of change in the beat interval. Dotted line indicates the stretch interval. Solid and open triangles correspond to those in A and B. C: Relationship between stretch phase and the change in the beat interval. Solid circles; plots from the first stretch to the 10th stretch. Open circles; plots from the 11th stretch to the19th stretch. Triangles correspond to those in A and B.

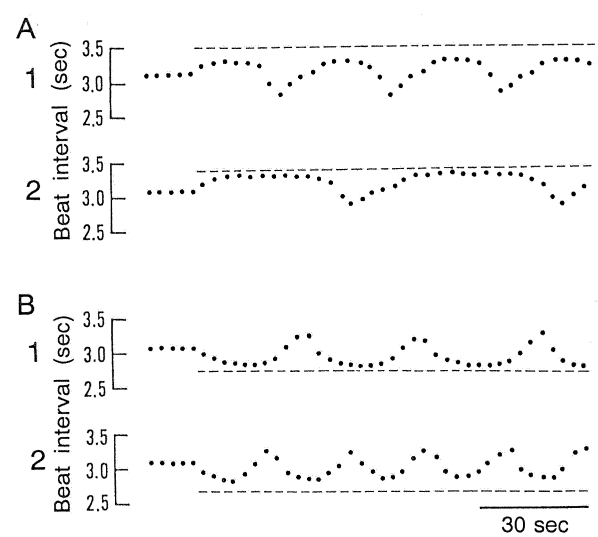

FIG. 9. Cyclical changes of the beat interval by repeated brief stretches in an isolated ventricle. Duration of stretch; 500 msec. Magnitude of stretch; 0.8 mm. Stretch interval; 3.50 in A1, 3.40 in A2, 2.80 in B1 and 2.70 sec in B2. Free-running beat interval; 3.13 to 3.17 sec. Dotted lines indicate intervals of the stretch applied.

entrainment to the same isolated ventricle. In both cases, the pattern of beat interval was repetitive and the period of the cyclical change was shorter with a lower (A1, A2) or higher stretch frequency (B1, B2).

DISCUSSION

Brief stretching of the isolated auricle or ventricle of the oyster delayed or advanced generation of the next beat and reset the beat rhythm (Fig. 1). Moreover, no significant difference in the PRC's for brief stretches was found between the auricle and ventricle (Fig. 2). These facts indicate that both the auricle and ventricle of the oyster possess properties which are characteristics of endogenous oscillators in general [12–14] and have the same phase-response characteristics for brief stretches.

Presence of the phase shift suggests that the beat might be entrained by repeated brief stretches [12, 14]. Beat frequency of the isolated auricle or ventricle was found to be entrained to a frequency of the repeated brief stretches, if the stretch frequency was not so much different from that of the free-running beat frequency (Fig. 4). When the stretch frequency was much lower or much higher, the beat frequency was not entrained, and changed cyclically instead. In both cases, the relations between each stretch phase and each change in the beat interval were consistent with that of the PRC (Figs. 6 and 8). These results strongly support the idea that the changes in the beat frequency by repeated brief stretches can be regarded as a process of repeated discrete phase shifts. This meant that entrainment of the beat frequency is achieved when each phase shift by each stretch becomes equal to the discrepancy between the stretch interval and the free-running beat interval. Detailed time courses of the entrainment clearly show that the beat frequency is entrained to the stretch frequency when the location of each stretch is fixed at the phase predicted from the PRC (Figs. 6 and 7). Thus, for a given stretch, entrainment of the beat frequency is achieved in the limited frequency range (range of entrainment) determined by the maximum values of phase delay and advance in the PRC. Since the maximum values of phase shift are larger with a larger stretch (Fig. 3), the range of entrainment is wider with larger stretches (Fig. 5).

When brief stretches were applied repetitively at a frequency outside the range of entrainment, the beat frequency was not entrained but changed cyclically (Fig. 4). Detailed time courses of the cyclical change clearly show that each beat interval changes according to cyclical change in the stretch phase, as predicted from the PRC (Fig. 8). With a higher or lower stretch frequency, a change in each stretch phase was greater and a period of cyclical change in the beat interval was shorter (Fig. 9).

In mammalian heart, synchronization of the beat rhythms between pacemaker cells is achieved by phase-response interaction (mutual entrainment) through electrical connections (e.g. [18]). In the molluscan heart in which any myocardial cells has endogenous automaticity [7, 8], rhythm synchronization among the myocardial cells is achieved by phase-response interaction through electrical connections [2–5]. However, in molluscan hearts, no electrical connection is found between the auricle and ventricle [16]. Coordinated beating of the molluscan heart is suggested to be achieved by stretch-mediated interaction between the auricle and ventricle [15–17].

As described above, the beat rhythm of the isolated auricle or ventricle of the oyster was entrained by repeated brief stretches according to its phase-response characteristics to brief stretches. This suggests that coordinated beating (alternate beating of the auricle and ventricle with the same frequency) of the oyster heart is achieved as a result of mutual entrainment of two endogenous oscillators, the auricle and ventricle, mediated by reciprocal stretching.

ACKNOWLEDGMENTS

We thank Dr. Y. Naitoh for helpful discussion and criticism of the manuscript. We also thank Dr. R. W. Ridge for reading the early draft of the manuscript. This is contribution No. 581 from the Shimoda Marine Research Center, University of Tsukuba.

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