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Phase-Response Analysis of Stretch-Mediated Beat Coordination in the Oyster Heart. II. Mutual Entrainment of Auricle and Ventricle

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ABSTRACT—Beat coordination between auricle and ventricle in the oyster heart was analyzed *in situ*. When the heart was exposed in the pericardial cavity, auricular and ventricular beats were not synchronized and the heart beat was irregular. In that state, the phase relationship between the auricular and ventricular beats changed successively and the beat frequency of both the auricle and ventricle changed cyclically. The relationship between stretch phase and beat interval change in the auricle and ventricle was identical with the phase response curve (PRC) of auricle and ventricle to brief stretches. By perfusing the pericardial cavity with sea water, the magnitude of reciprocal stretching between auricle and ventricle increased gradually. Then the auricle and ventricle began to beat synchronously with a fixed phase relation and coordinated beating of the heart was achieved. These results strongly support the idea that a coordinated heart beat of the oyster is achieved as a result of mutual entrainment of the auricle and ventricle mediated by reciprocal stretching.

INTRODUCTION

In the preceding paper, we showed that both the auricle and ventricle of oyster have the same phase-response characteristics to brief stretches and their beat rhythms can be entrained by repeated brief stretches. These results have suggested that coordinated beating of the oyster heart is achieved by mutual entrainment [5, 9] of the auricle and ventricle mediated by reciprocal stretching.

To demonstrate the idea, we attempted to examine the oyster heart beat *in situ*. When the heart is exposed *in situ*, in the pericardial cavity, coordination between auricle(s) and ventricle is lost and the heart beats irregularly. By perfusing the pericardial cavity with sea water, the coordination is restored and the heart beats with a sustained regular rhythm [7]. We therefore examined the phase relations between each stretch and each beat in the auricle and in the ventricle in an uncoordinated state of the heart beat under non-perfused conditions. We also examined the precise time course of changes in the magnitude of reciprocal stretching between the auricle and ventricle and in the beat interval in the auricle and ventricle when achieving the coordinated heart beat under perfused conditions.

MATERIALS AND METHODS

The experiments were performed on semi-intact hearts of the Japanese oyster *Crassostrea gigas*. After removing the upper (right) valve, the heart was exposed by removing the upper wall of the pericardial cavity. Nerves from the visceral ganglion were cut to eliminate neural effects on the heart. Arteries and veins were also cut to eliminate effects of blood flow on the heart beat. One of the two auricles was removed from the heart to make the analysis of the mechanical interaction between auricular and ventricular beats easier. The pericardial cavity was perfused with aerated artificial sea water (ASW) to maintain coordinated heart beating. Auricular and ventricular beats were monitored with a mechano-electric transducer placed between the auricle and ventricle (mechanogram of the heart beat). The auricular beats produced positive signals and the ventricular beats negative signals. Auricular and ventricular action potentials, which accompany the beats, were simultaneous recorded through two suction electrode, one placed at the venous end (auricular action potential) and the other at the aortic end (ventricular action potential). The electrodes were always under suction by using electric suction-pumps. The shape of the action potential somewhat changed during experiments, probably in part due to the movement of the tissue, the beat interval (from the peak of one action potential to that of the next) could always be determined in the auricle and in the ventricle.

Details of the electrical recording and composition of ASW are described in the preceding paper. All the experiments were performed at a temperature of 20 to 24°C.

RESULTS

Uncoordinated heart beat under non-perfused conditions

When the heart of oyster is exposed *in situ*, in the pericardial cavity, by removing the pericardial wall, coordination between the auricle and ventricle is lost and the heart beats irregularly. Under such conditions, the precise time course of changes in the beat interval in the auricle and in the ventricle was examined. Figure 1 shows an example. Action potentials were simultaneously recorded from the auricle and ventricle (A). To visualize beat interval changes, the interval between each action potential peak is plotted against time (B). In this case, the beat interval of the ventricle was always higher than that of the auricle. Therefore, the phase

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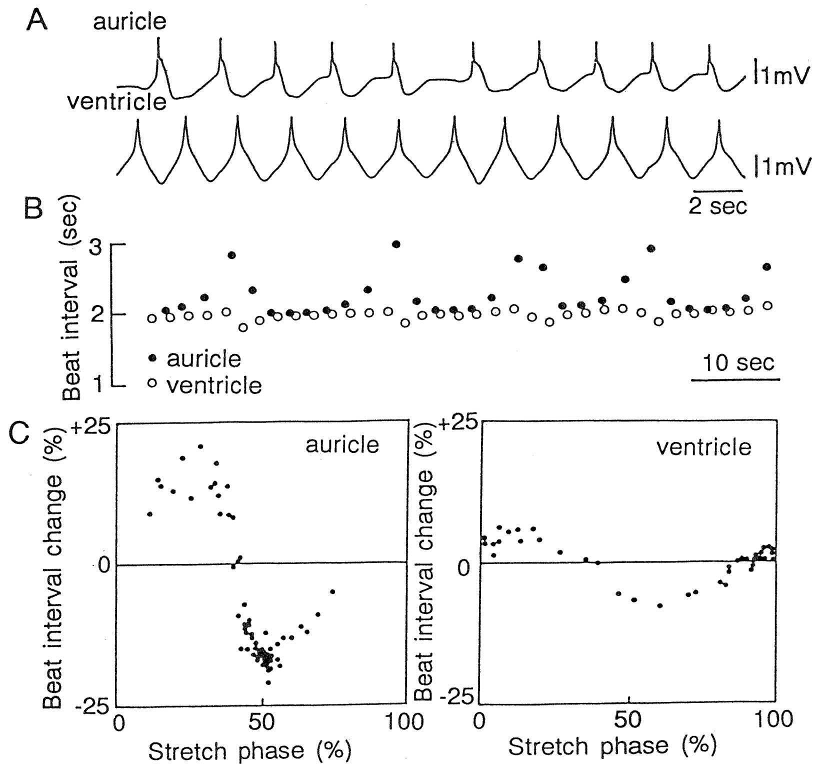

Fig. 1. Phase relationship between repeated brief stretches of auricle (ventricle) by ventricular (auricular) beats in uncoordinated heart under non-perfused conditions. A: Action potentials recorded extracellularly from the auricle (upper trace) and from the ventricle (lower trace). B: Time course of change in the beat interval of the auricle (solid circles) and of the ventricle (open circles). C: Relationship between the stretch phase and the change in the beat interval of the auricle (left) and of the ventricle (right).

relationship between the auricular and ventricular action potential peak changed successively (A), and the beat interval of the auricle and ventricle changed cyclically (B). The range of beat interval change was much wider in the auricle than in the ventricle (B).

We have shown in the preceding paper that a change in the beat interval of the auricle or ventricle by its brief mechanical stretching is dependent on the stretch phase (the moment when the stretch is applied to the tissue). In the heart preparation, the beat of the ventricle apparently stretches the auricle and *vice versa*. Therefore, the relationship between stretch phase and change in the beat interval was examined in the auricle and in the ventricle. The control beat interval was defined as a mean value of the maximum and minimum beat intervals observed in six successive cyclical changes, and the percent change in each beat interval relative to the control was determined. The stretch phase of the auricle by the ventricle was defined as the period of time from the preceding auricular action potential peak to the peak of ventricular contraction (600 msec after the ventricular action potential peak). The stretch phase of the ventricle by the auricle was defined as that from the preceding ventricular action potential peak to the peak of auricular contraction (500 msec after the auricular action potential peak). The stretch phase was expressed as a percentage of the control beat interval. The change in the beat interval was plotted against the stretch phase. As shown in Figure 1C, the relationship in both the auricle (left) and ventricle (right) is essentially identical with the PRC to brief stretches obtained in an isolated auricle and ventricle.

Coordinated heart beat by pericardial perfusion

The heart of the oyster exposed *in situ* exhibited coordinated beating by perfusing the pericardial cavity with ASW. As shown in Figure 2A, the mechanogram of the heart (upper trace) was simultaneously recorded with the auricular (middle trace) and ventricular (lower trace) action potentials. The coordination between the auricle and ventricle was maintained as long as the pericardial cavity was perfused. The auricle and ventricle beat with the same frequency, keeping a fixed phase relation as seen in the mechanogram (see left faster recording portions), where auricular and ventricular beats are shown as upper (positive) and lower (negative) deflections respectively. Since the auricle and ventricle stretched each other by their contractions, the amplitude of the mechanogram during one heart beat cycle (from an auricular contraction to the following ventricular contraction) was defined as the magnitude of reciprocal stretching between auricle and ventricle.

When the perfusion was stopped (down arrow in Fig. 2A), the magnitude of reciprocal stretching decreased gradually and the coordinated heart beat was lost. As shown in Figure 2B1, the phase relationship between the auricular and ventricular beats changed successively, and the amplitude and

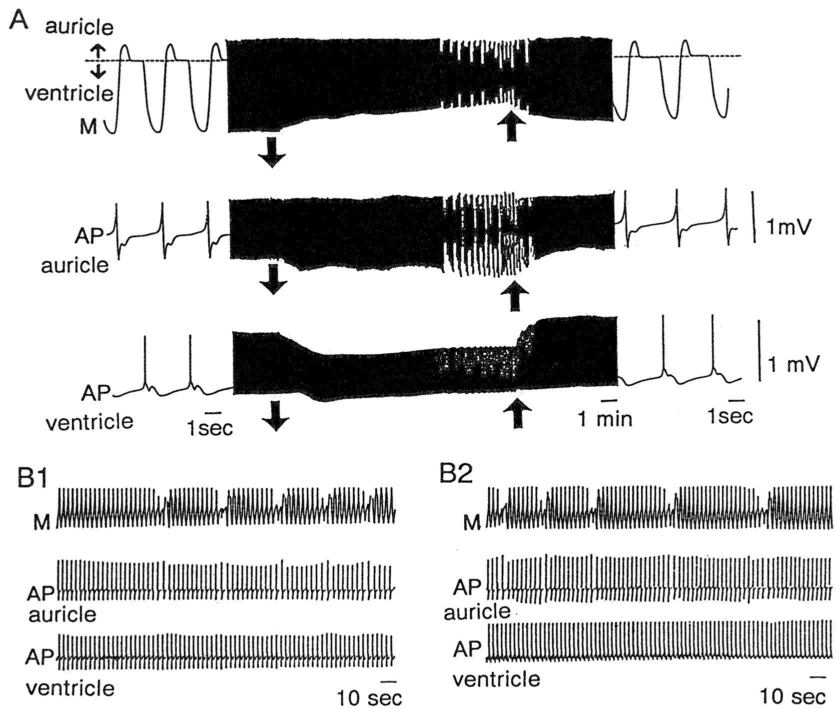

FIG. 2. Effects of pericardial perfusion on the oyster heart beat *in situ*. A: Simultaneous recording of mechanogram of the heart beat (M, top trace) and extracellular action potentials (AP) from the auricle (middle trace) and ventricle (bottom trace). Upward and downward deflections in the mechanogram correspond to auricular and ventricular contractions, respectively. Dotted line indicates a reference level. Downward and upward pointing arrows show the moment when the pericardial perfusion stopped and the moment when it was resumed, respectively. B: Faster records after stopping (1) and resuming (2) the perfusion. The same data as in A.

baseline of the mechanogram changed periodically. When the perfusion was resumed (upper arrow in Fig. 2A), the magnitude of reciprocal stretching increased gradually and the coordinated beat was progressively restored (Fig. 2B2).

Figure 3 shows the time course of changes in the magnitude of reciprocal stretching and in the beat interval of the auricle and ventricle after stopping (A) and resuming (B) the perfusion. In the uncoordinated state of the heart beat, the magnitude of reciprocal stretching was determined at the time when a phase relation between the auricular and ventricular beats was almost the same as that in the coordinated state (control state) and expressed as a percentage of the control magnitude (top trace). Each beat interval of the auricle (middle trace) and ventricle (lower trace) was successively plotted against time. After stopping the perfusion (A), the auricular and ventricular beats began to change cyclically when the magnitude of reciprocal stretching decreased to less than 90% of the control. The period of cyclical change of the beat interval became shorter as the magnitude of reciprocal stretching decreased.

After resuming the perfusion (Fig. 3B), the magnitude of reciprocal stretching increased gradually and the period of cyclical change of the auricular and ventricular beat intervals became longer. When the magnitude increased to about 90% of the control a fixed phase relation was established between the auricular and ventricular beats (cf. right faster recording portions in Fig. 2) and the coordinated heart beat was achieved.

DISCUSSION

In the mollusca, the pericardium surrounded by a tough pericardial membrane has a fixed volume and functions in the refilling of the heart [4, 6]. When the pericardium is opened, the heart beat is seriously impaired [1]. On the other hand, the isolated heart loses a coordinated beat, but exhibits a sustained regular beat when stretched [2, 3]. In the oyster, the heart loses the coordinated beat when the pericardium was opened, but the coordination was restored by perfusion of the pericardial cavity with sea water. Though the reason why the coordinated heart beat was achieved by pericardial perfusion is still uncertain, it might exert the effect on the heart as of continuous stretching.

The auricle and ventricle stretched each other by their contractions. In uncoordinated state of the heart beat under non-perfused conditions, the relationship between stretch phase and beat interval change of both the auricle and ventricle was essentially identical with the PRC for brief stretches obtained in the preceding paper (Fig. 1C). This suggests that the auricle and ventricle interact with each other by mutual stretching between them according to their phase-response characteristics to brief stretches. In the heart preparation in which one of the two auricles was removed and used in this study, the stretch by the ventricular contraction was much greater than that by the auricular contraction. Therefore, as predicted from the phase-response characteristics that a phase shift is larger with a larger stretch, the range

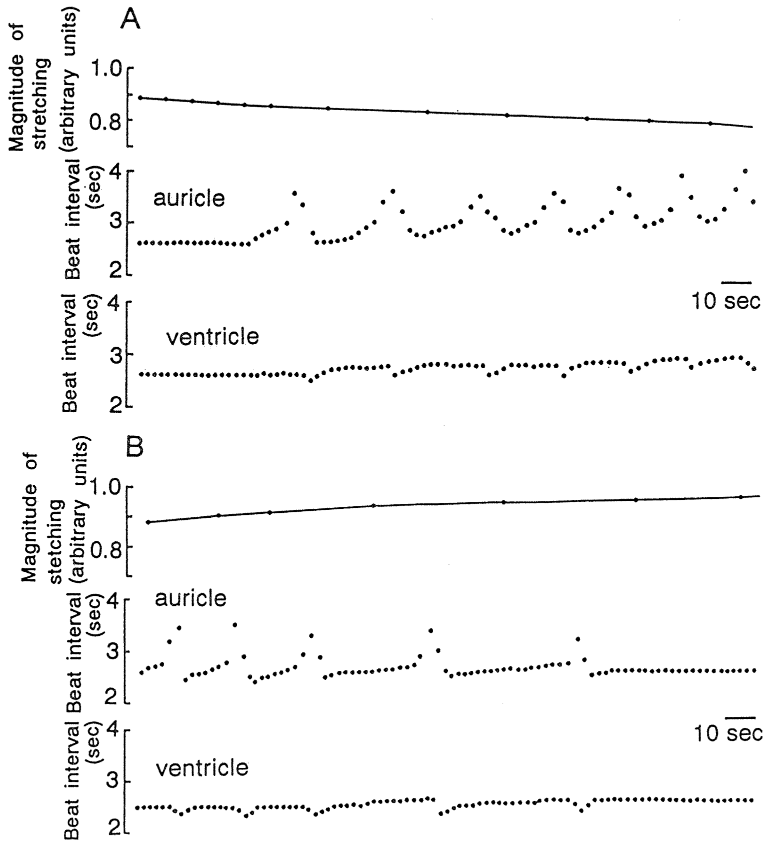

Fig. 3. Time course of changes in the magnitude of reciprocal stretching between auricle and ventricle and in the beat interval of the auricle and ventricle after stopping (A) and resuming (B) the pericardial perfusion. The magnitude of reciprocal stretching (stretch magnitude; upper trace) and the change in the beat interval of the auricle (middle trace) and ventricle) (bottom trace) are plotted against time. The same data as in Fig. 2.

of beat interval change was much wider in the auricle than in the ventricle (Fig. 1 A, B). Moreover, the beat interval of the auricle and ventricle changed cyclically (Fig. 1 A, B) as an isolated auricle or ventricle subjected to repeated stretches with a frequency outside the range of entrainment.

The above results also suggest that the auricle and ventricle can be entrained with each other (mutual entrainment) [5, 9] by a greater magnitude of reciprocal stretching between them. By perfusing the pericardial cavity with sea water, the magnitude of reciprocal stretching increased gradually and a coordinated heart beat was achieved (Fig. 2A). As the magnitude of reciprocal stretching increased, the period of cyclical change in the auricular and ventricular beat intervals became longer. Then a fixed phase relationship between the auricular and ventricular beats was established at a certain value of the stretch magnitude and coordinated heart beat was achieved (Figs. 2B2 and 3B). Contrastingly, after stopping the perfusion, the magnitude of reciprocal stretching decreased gradually and the coordinated heart beat was lost (Fig. 2A). As the magnitude of reciprocal stretching decreased, the auricular and ventricular beat interval began to change cyclically and the period of the cyclical change became shorter (Figs. 2B1 and 3A). In the preceding paper, it was shown that the range of entrainment by repeated brief stretches is wider with a larger stretch and, under conditions outside of the range of entrainment, the period of cyclical change in the beat interval is shorter with higher or lower stretch frequency. Therefore, the relationship between the changes in the stretch magnitude and in the period of cyclical beat interval change after starting or stopping the perfusion could be well understood from the phase-response characteristics of the auricle and ventricle to brief stretches.

From the results described above it can be concluded that the beat coordination between the auricle and ventricle of the oyster is achieved by mutual entrainment mediated by their reciprocal stretching. This also means that a regular sustained beat rhythm of the oyster heart is determined as a result of stretch-mediated phase-response interaction between the auricular and ventricular beat rhythms.

In the heart of the oyster, two auricles couple electrically and fire synchronously [8]. Therefore, in an intact heart, stretch of the ventricle by auricular contraction is greater than in the heart from which one of the two auricles was removed. This suggests that mutual entrainment of the beat rhythms between auricles and ventricle occurs more easily in the intact heart than in the heart preparation used in this study.

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