Coupling interval from slow to tachycardiac pacing decides sustained alternans pattern

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Various myocardial conditions are known to affect the pattern and amplitude of the mechanical alternans. Cardiac anoxia, ischemia (27, 34), tachycardia (4), and hypothermia (16) cause or intensify sustained mechanical alternans. Administration of epinephrine (4), caffeine (12, 13, 25), and ryanodine (13) diminish or eliminate the alternans. On the other hand, the same alternans pattern reappeared even after a premature or delayed beat interrupting the sustained alternans (17, 29). These results have yielded a general view that the pattern and amplitude of sustained mechanical alternans would change with cardiac contractile conditions at the same beating rate.

However, we accidentally observed that the interbeat alternation pattern and amplitude of the sustained contractile alternans induced by tachycardiac pacing varied considerably with a single coupling beat interval between the same slow and tachycardiac pacing periods. Intriguingly, sustained contractile alternans even disappeared after a specific coupling interval. No literature has documented these observations.

Therefore, we investigated the relationship between the coupling interval and the interbeat alternation pattern and amplitude of sustained contractile alternans in the excised, cross-circulated canine heart. We produced sustained contractile alternans by an abrupt increase in regular pacing rate in normal canine hearts. Our results showed that 1) the alternans pattern and amplitude changed markedly with the coupling interval in all of the hearts, 2) the alternans disappeared at 1–3 specific coupling intervals in each heart, and 3) the even- and odd-numbered order of strong and weak beats counted from the coupling interval reversed across these specific coupling intervals. These findings indicate that a more severe sustained contractile alternans under a given tachycardiac pacing is not always indicative of a worse cardiac contractile condition.

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METHODS

Surgical preparation. We performed the present experiments in the canine excised, cross-circulated (blood perfused) heart preparation that we have been using consistently in our laboratory (3, 14, 18, 24, 28, 31). We conducted them in conformity with the “Guiding Principles for Research Involving Animals and Human Beings,” endorsed by the American Physiological Society. The surgical procedures were described in detail elsewhere (14, 18, 24). Briefly, two mongrel dogs (9–23 kg body wt) were anesthetized with pentobarbital sodium (25 mg/kg iv) after premedication with ketamine hydrochloride (20 mg/kg im). Anesthesia was maintained by fentanyl (200–250 μg/kg iv). The dogs were intubated and artificially ventilated and were then heparinized (10,000 units iv per dog).

We used the larger dog (18 ± 3 kg body wt; means ± SD) as the metabolic supporter. Its common carotid arteries and right external jugular vein were cannulated and connected to the arterial and venous cross-circulation tubes, respectively. The chest of the smaller dog (12 ± 2 kg body wt) was opened midsternally, and the heart was used as a donor. The arterial and venous cross-circulation tubes were cannulated into the left subclavian artery and the right ventricle through the right atrial appendage, respectively, of the donor dog. In each of the 12 experiments, the heart-lung section was isolated from the systemic and pulmonary circulation by ligating the azigos vein, descending aorta, inferior vena cava, brachiocephalic artery, superior vena cava, and bilateral pulmonary hilii. The beating heart, supported by cross circulation, was then excited from the chest. Coronary perfusion of the excised heart was never interrupted during the preparation. We gave indomethacin (5–20 mg iv; donated by Banyu Pharmaceutical; Tokyo, Japan) to the support dog to prevent systemic hypotension occasionally elicited by blood cross circulation (18, 24).

The left atrium was opened, and all of the left ventricular (LV) chordae tendineae were cut. Complete atroventricular block was made by electrical ablation or by injecting 40% formaldehyde through the right atrium into the region of the His bundle (30). Electrical pacing was performed with a bipolar electrode placed on the upper interventricular septum. A thin latex balloon (unstressed volume, ~50 ml) mounted on a rigid connector was fitted into the LV, and the connector was secured at the mitral annulus. LV pressure was measured with a miniature pressure gauge (model P-7, Konigsberg Instruments; Pasadena, CA) inside the apical end of the balloon and processed with a direct current strain amplifier. The balloon, primed with water without air bubbles, was connected to our custom-made volume servo pump (AR-Brown; Tokyo, Japan). LV volume was accurately controlled and precisely measured with the servo pump. LV epicardial electrocardiogram (ECG) was recorded with a pair of screw-in electrodes to trigger data acquisition and identify the onset of contraction.

We monitored and maintained cardiac temperature in an acrylic box by warming the coiled portion of the arterial cross-circulation tube in a thermostat bath. In our preliminary experiment, we needed a tachycardiac pacing rate of 250–300 beats/min to produce sustained contractile alternans at 36–37°C. This suggests that the heart we prepared was reasonably physiological (34). This condition, however, rapidly deteriorated the contractility of the heart, probably because the tachycardia-induced increase in oxygen demand exhausted the coronary reserve (31). Therefore, we kept cardiac temperature slightly hypothermic (34.2 ± 1.0°C) to produce stable sustained contractile alternans at a pacing rate under 250 beats/min in the present study.

Mean systemic arterial blood pressure of the support dog (118 ± 16 mmHg) served as coronary perfusion pressure. It was maintained stable in each experiment by slowly trans-fusing whole blood reserved from the heart donor dog or by infusing 6% hydroxyethyl starch solution and by continuously infusing methoxamine (5–30 mg/h) as needed. We measured repeatedly arterial pH (7.42 ± 0.07), Po2 (131 ± 32 mmHg), and Paco2 (31 ± 8 mmHg) of the support dog and normalized them with supplemental oxygen and intravenous NaHCO3 as needed.

Pacing stimuli. We programmed the pacing stimuli with the use of LabView, version 3.1 (National Instruments), on a Power Mac computer, which controlled a stimulator with an analog-to-digital converter (Lab-NB, National Instruments).

Figure 1 shows two representative set of pacing stimuli in one heart. We provided a priming period of regular slow pacing stimuli at intervals of 500 ms (~120 beats/min) in 9
hearts and 450 ms (133 beats/min) in the other 3 hearts. We then provided a tachycardiac period of regular pacing stimuli at shorter intervals of 283 ± 24 ms (~213 ± 18 beats/min) to generate sustained contractile alternans. We provided a variable coupling interval between the slow priming and tachycardiac periods as a sole independent variable. We performed similar runs only by changing the coupling interval in steps of 20–50 ms from 200 to 600 ms in each heart.

Experimental protocol. We used isovolumic LV contractions throughout this study. Our preliminary experiment showed that the LV with a large isovolumic volume could not maintain stable sustained contractile alternans. We eliminated this problem by keeping LV isovolumic volume at a midrange volume of 12.3 ± 2.4 ml (89 ± 18 g wt), where LV peak isovolumic pressure was <100 mmHg.

We continuously monitored LV pressure through the slow and tachycardiac pacing periods and recorded it during the last several slow beats, and both the transient and stable sustained contractile alternans beats for 1–1.5 min. We then returned to the priming period with slow pacing until LV contractility became stable. Each experiment was completed within a few hours while the condition of the preparation was stable. In three experiments, we repeated the entire protocol to check the reproducibility of the results.

Monophasic action potential. Monophasic action potential (AP) was recorded simultaneously with a contact electrode catheter (7) (Langendorff probe, Boston Scientific) on the anterolateral LV surface near its obtuse margin in three hearts. We measured the duration of the monophasic AP duration at the 90% repolarization (APD90) from the onset of the steep upstroke to the 90% repolarization level. Here, 100% was defined as the height of the monophasic AP from its diastolic baseline to the crest of its plateau (10). We measured APD90 in six consecutive beats during stable sustained contractile alternans. The six APD90 values were averaged for three strong and three weak beats separately.

Data analyses. LV pressure, volume, ECG, and APD signals were digitized at 2- or 4-ms intervals with an analog-to-digital converter (Lab-NB, National Instruments), displayed, and stored on the hard drive of a computer.

Statistics. Data were presented as means ± SD. Comparisons of the parameters between the strong and weak beats were made by Student’s paired t-test. A value of P < 0.05 was considered significant.

RESULTS

Sustained alternans. Figure 1 shows simultaneous tracings of LV pacing stimuli, ECG, and isovolumic pressure in two representative runs in one heart. These two runs had the same set of the slow pacing interval (500 ms) for the priming period and the tachycardiac pacing interval (300 ms) but two different coupling beat intervals (400 and 600 ms). Under tachycardia, the contractile alternans changed transiently over the first 10–15 s or 30–50 beats. Thereafter, the strong and weak alternation pattern and amplitude of the peak isovolumic pressure alternans and hence contractile alternans became gradually stable over 1 min.

The peak pressure alternated remarkably during the initial transient and successive stable periods of tachycardiac pacing in Fig. 1A, whereas it stopped to alternate after the initial transient period of tachycardiac pacing in Fig. 1B. Similar results were obtained in all of the hearts. The index of LV contractility, E′max, was reasonably good (8.3 ± 3.8 mmHg · ml⁻¹ · 100 g⁻¹) (14, 24, 28, 31, 32). Here, E′max was calculated as LV peak isovolumic pressure divided by LV volume minus the initial volume obtained as the LV volume at which peak isovolumic pressure was zero (31, 32). We confirmed occasionally that the sustained contractile alternans continued stably with the same pattern and amplitude of interbeat alternation over 10 min.

The valley, or end-diastolic pressure, also alternated during the contractile alternans. The higher valley pressure followed the higher peak pressure and the lower valley pressure followed the lower peak pressure.

Coupling interval and alternans. Figure 2 shows representative relationships between the coupling interval and the peak isovolumic pressures of the strong and weak beats in stable sustained contractile alternans. LV contractilities of the alternans beats were proportional to these peak pressures because LV volume was constant. Figure 2A shows that both peak and valley pressures of the alternans beats changed sensitively with coupling interval. However, the alternans of both peak and valley pressures completely or almost disappeared at coupling intervals of 210, 300, and 600

![Fig. 2. Relationship between CI and the alternating peak and valley pressures of the strong and weak beats of the sustained contractile alternans.](http://ajpheart.physiology.org/Downloadedfromhttp://ajpheart.physiology.org/)
ms (arrows on the abscissa). Furthermore, the order of the strong and weak beats counted from the coupling interval reversed across a coupling interval of 300 ms. Above 300 ms, the odd-numbered beats were stronger and the even-numbered beats were weaker. Below 300 ms, the odd-numbered beats were weaker and the even-numbered beats were stronger.

Figure 2B shows that the alternans amplitude of both peak and valley pressures suddenly diminished at a coupling interval of 375 ms (arrow) and the alternans order reversed across this coupling interval. At the other coupling intervals, the alternans amplitude of both peak and valley pressures remained little changed with the coupling interval. The alternans amplitude remained little changed, particularly between 250 and 360 ms.

These results in Fig. 2 show the existence of at least one specific coupling interval in each heart, at which the amplitude of sustained contractile alternans totally or almost disappeared and across which the alternans order of strong and weak beats reversed. Such a specific coupling interval existed between the slow priming and tachycardiac pacing intervals, which are indicated by the two dashed lines in Fig. 2. We obtained similar results in all of the hearts. The order of strong and weak beats at the longer or shorter interval than a specific coupling interval was uncertain among hearts. Unless we counted carefully the beat number after the coupling interval, we could not distinguish the sustained contractile alternans of similar patterns and amplitudes at longer and shorter intervals than the specific coupling interval.

Table 1 summarizes the number of the specific coupling intervals and the incidence of different types of the relationship between the coupling interval and the alternation pattern in all of the hearts. We counted the number of the coupling intervals at which the alternans disappeared or the order of the strong and weak beats reversed. We classified these relationships into two types. Type I was that the alternans amplitude of both peak and valley pressures continuously changed between the specific coupling intervals as shown in Fig. 2A. Type II was that the alternans amplitude was relatively stable at all of the coupling intervals except around the specific coupling intervals as shown in Fig. 2B.

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Fig. 3. A–F: transition from the transient to sustained contractile alternans after various CI in one left ventricle. Left: LVP from slow beats to the 50th beat from the first tachycardiac beat in slow-speed recording. Right: LVP of the 201st and 202nd beats in high-speed recording. L, slow beat interval; H, tachycardiac pacing interval.
first increased from Fig. 3, A–C, then decreased from Fig. 3, C and D, and increased again from Fig. 3, D–F. The alternans almost disappeared, as shown in Fig. 3 D, namely, at a specific coupling interval. Between Fig. 3, D and E, the order of the strong and weak beats reversed as exemplified in the odd- (o, 201st) and even-numbered (e, 202nd) beats. We obtained similar results in all of the hearts.

The valley pressure alternans was largely proportional to the peak pressure alternans in the transient phase of alternans. However, as the peak pressure alternans became stable, the valley pressure alternans almost or completely disappeared, as shown in Fig. 3, A, D, and E, but remained a little in Fig. 3, B, C, and F. Therefore, a higher valley pressure did not always precede a proportionally weaker peak pressure in the next beat.

**Action potential duration.** We found that monophasic AP showed little alternans during the sustained contractile alternans. The APD<sub>90</sub> of the strong and weak beats recorded in stable sustained contractile alternans were 211 ± 3 versus 212 ± 3 ms (12 runs) in one heart, 210 ± 6 versus 209 ± 4 ms (8 runs) in another heart, and 192 ± 5 versus 193 ± 5 ms (13 runs) in a third heart. The difference in APD<sub>90</sub> between strong and weak beats was not significant in these hearts (Student’s paired t-test, P > 0.05).

**DISCUSSION**

**New finding.** The most important finding in this study is that the pattern and amplitude of the strong and weak contractions in sustained contractile alternans varied markedly with the coupling interval between the same set of slow priming and the tachycardiac pacing periods. Although previous investigators have produced sustained contractile alternans by abruptly increasing the pacing rate (4, 9, 25, 26, 34, 36), no one has recognized the importance of the coupling interval between the slow priming and tachycardiac pacing periods. We obtained the present finding while we maintained stable the cardiac contractile conditions that could affect the severity of sustained contractile alternans (4, 16, 27, 34).

**Ca<sup>2+</sup> handling.** The sustained contractile alternans must be the result of alternating contractility, because we fixed LV volume to exclude the Starling effect. We speculate that the contractility alternans reflects alternans of myocardial Ca<sup>2+</sup> handling (6, 28, 35, 36). Even an extrasystole affects myocardial Ca<sup>2+</sup> handling in successive beats as manifested by postextrasystolic restitution and potentiation (3, 6, 23, 28). These postextrasystolic effects involve two Ca<sup>2+</sup> handling mechanisms. One is a change in transsarcolemmal Ca<sup>2+</sup> influx in the extrasystole and its gradual recovery over successive beats (23, 28). The other is a change in Ca<sup>2+</sup> recirculation (i.e., uptake and release) via the sarcoplasmic reticulum (6, 22, 28). However, these changes disappear after several postextrasystolic beats under pacing at a slow enough rate not to produce sustained contractile alternans (6, 28, 35, 36). However, sustained contractile alternans occurs by sufficiently tachycardiac pacing even in normal hearts (34, 36). One or both transsarcolemmal and sarcoplasmic Ca<sup>2+</sup> handling mechanisms seem responsible for the sustained contractile alternans that we observed.

Because there was no significant alternation of APD<sub>90</sub>, alternation of the monophasic AP, and hence transsarcolemmal Ca<sup>2+</sup> influx, are not likely to be a main cause of the sustained contractile alternans in the present study. In intact hearts, a Ca<sup>2+</sup> channel blockade, verapamil, suppressed APD alternans but kept LV pressure alternans (12). This suggests that sustained contractile alternans require sarcoplasmic reticulum Ca<sup>2+</sup> handling, but not always transsarcolemmal Ca<sup>2+</sup> influx alternans. This is compatible with the finding of no significant APD<sub>90</sub> alternans during the sustained contractile alternans in the present study.

We suspect a mechanism to exist by which even a single coupling interval preceding the tachycardiac pacing decides the pattern and amplitude of the sustained contractile alternans. Even a single coupling interval affects the excitation-contraction coupling and the contraction of the several postextrasystolic tachycardiac beats. This effect in turn may continuously affect the sustained contractile alternans. As shown in Figs. 1 and 3, transient contractile alternans began tachycardiac pacing. The alternans waxed and waned for the initial 10–15 s or 30–50 beats. The greater amplitude the transient contractile alternans had, the greater amplitude the stable contractile alternans had. At a specific coupling interval, the amplitudes of both transient and sustained contractile alternans were smaller than at other coupling intervals. This suggests that the alternans amplitude initiated on the tachycardiac pacing is carried over to the successive alternans beats without fading out. This maintenance means that a mechanism for a stronger beat follows a weaker beat, despite the regular beat intervals under tachycardia. This mechanism itself is essentially what maintains sustained mechanical alternans under tachycardia in normal hearts (34).

**Simulation.** We examined whether any sustained-contractile-alternans model on the basis of myocardial Ca<sup>2+</sup> handling in the literature could simulate our present finding. We first used Adler et al.’s model (1, 2). This model incorporated two Ca<sup>2+</sup> handling mechanisms (1, 2). One is a Ca<sup>2+</sup> release to myofilaments on depolarization as a function of Ca<sup>2+</sup> in a releasable terminal to affect only the subsequent beat. The other is a strong Ca<sup>2+</sup> buffering capability of the sarcoplasmic reticulum to affect several subsequent beats. This model also incorporated transmembrane Ca<sup>2+</sup> influx and efflux. The Adler model successfully simulated sustained contractile alternans with an abrupt increase in the beating rate, as shown in Fig. 4. Although a step increase in heart rate from 120 to 240 beats/min generated sustained contractile alternans, the pattern and amplitude of the contractile alternans remained identical despite varied coupling intervals (200–400 ms). Although we recognized that the order of strong
and weak beats reversed between coupling intervals of 300 and 400 ms, no coupling interval existed to attenuate or eliminate the alternans. The Adler model could not simulate any diastolic pressure alternans.

Freeman et al. (8) presented a different model to simulate sustained contractile alternans. They used three time constants to explain the \(\text{Ca}^{2+}\) movement between the three compartments, i.e., the sarcoplasm, a \(\text{Ca}^{2+}\) uptake pool, and a \(\text{Ca}^{2+}\) releasable pool (38). They assumed the \(\text{Ca}^{2+}\) uptake time constant to be inversely proportional to sarcoplasmic \(\text{Ca}^{2+}\) (35). However, they assumed no contribution of transsarcolemmal \(\text{Ca}^{2+}\) transport to the alternans, namely, constancy of the total intracellular \(\text{Ca}^{2+}\) among alternans beats. Figure 5 shows our simulation using this Freeman model. Figure 5A shows sustained contractile alternans generated even by a single extrasystole interrupting the continuous regular beats (400 ms). These extrasystoles after coupling intervals of 250 (upper) and 300 ms (lower) generated different but stable patterns of alternation of sarcoplasmic \(\text{Ca}^{2+}\). However, we were not able to produce this type of nontachycardiac sustained alternans in our heart preparation. Figure 5B shows our simulation of sustained contractile alternans during tachycardia in a protocol similar to our experiment. These coupling intervals of 250 and 500 ms generated sustained contractile alternans and the pattern and amplitude of sarcoplasmic \(\text{Ca}^{2+}\) alternans depend on the coupling interval. However, the alternans waxed gradually after both coupling intervals until the strong beat increased twofold of the regular beat and the weak beat faded out completely. Thus the Freeman model as it is cannot simulate our present observation. This model can neither yield any obvious end-diastolic \(\text{Ca}^{2+}\) alternans. This may be due to the assumed constancy of the total intracellular \(\text{Ca}^{2+}\) throughout the transient and steady-state phases of tachycardia.

A new \(\text{Ca}^{2+}\) handling model, including an appropriate combination of the Adler et al. (1) and Freeman et al. (8) models, may account for the present finding. Development of such a model is beyond the scope of the present study, although such an effort is warranted. The Adler and Freeman models do not include alternating changes in end-diastolic (valley) pressure and \(\text{Ca}^{2+}\). Additional components like the end-diastolic pressure and \(\text{Ca}^{2+}\) alternans to these models may help simulate our observation of the valley pressure alternans accompanying the peak pressure (contractile) alternans. The Adler and Freeman models included neither alternating changes in \(\text{Ca}^{2+}\) sensitivity nor responsiveness of contractility. A recent study (37) suggests that there is an interbeat change in the relation between \(\text{Ca}^{2+}\) handling and contractility after an extrasystole. The addition of such a mechanism to the Adler and Freeman models might lead to a successful simulation, although we have not attempted this.


**Limitations.** One may expect that inserting an appropriately coupled extrasystole could abolish sustained alternans. If this method works, it could be used to abolish alternans. Although this possibility remains to be studied, the disappearance of alternans per se may not be beneficial as long as tachycardia remains. Besides, because we studied isovolumic contractions in the excised heart, it remains unknown whether the same phenomenon could occur in ejecting contractions in situ hearts. Because we used normal hearts, the same phenomenon remains to be examined in failing hearts.

In summary, our new finding is the coupling interval-dependent variation of the pattern and amplitude of the sustained contractile alternans. We were not able to simulate this observation with the representative Ca²⁺ handling models reported in the literature. The cellular mechanism of the phenomenon remains to be elucidated for better understanding of cardiac sustained contractile alternans and Ca²⁺ handling.

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