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A study on premature ventricular contractions caused by ultrasound exposure with microbubbles using cultured ventricular muscle cells

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Abstract. It has been shown that diagnostic ultrasound examination using a contrast agent can cause premature ventricular contractions (PVCs). In this study, we investigated a usefulness of a new technique using cultured cardiac myocytes to study mechanisms of PVC production. Cardiac myocytes were isolated from neonatal rats and cultured on a cover glass. The cover glass was attached to an observation chamber in which it was possible to observe changes in myocytes during ultrasound exposure. In the experiments, cardiac myocytes were exposed to pulsed ultrasound in the presence and absence of microbubbles. The pressure amplitudes (peak-negative pressures) were set at 5 steps, —0.28, —0.55, —0.73, —0.92 and —1.1 MPa, and threshold pressure to produce a PVC was recorded. The results showed that the presence of microbubbles attached to a cell reduces threshold pressure for producing PVCs, and it was concluded that our method is useful for studying the mechanisms of PVC production.

1. Introduction
Ultrasound contrast agents (UCAs) are widely used now, but the biological effects of UCAs have become a matter of great concern. Production of premature ventricular contractions (PVCs) is known to be one of the side effects of contrast echocardiography. The dependency of PVC production on exposed ultrasound pressure has been investigated by in vivo studies using human volunteers [1] and experimental animals [2], and it has been shown that the probability of PVC production increases above a certain pressure threshold. Elucidation of the mechanism of PVC production is important to establish a safe method for using UCAs; however, it is difficult to observe myocyte damage in situ.
In this study, we investigated the possibility of using cultured cardiac myocytes isolated from neonatal rats to study the mechanisms of PVC production.

2. Methods
Cultured cardiac myocytes of neonatal rats start autonomous pulsation several days after isolation [3]. Although the myocytes have no automaticity in vivo, isolation of the myocytes gives them automaticity. In this study, cardiac myocytes of neonatal rats were used for in vitro experiments, and changes in cyclic contraction rhythm caused by pulsed ultrasound exposure in the presence and absence of microbubbles were observed.

Cardiac myocytes isolated from neonatal rats were incubated on a cover glass of 0.16 mm in thickness. Slight movement of cyclic contraction was observed in some myocytes two or three days after start of incubation (Figure 1(a)). Five to seven days after the start of incubation, aggregation occurred in myocytes as shown in Figure 1(b), and synchronous contraction was observed. Cardiac myocytes at this stage were used as an observation sample in this experiment.

Figure 2 shows the observation system used in this study. An inverted microscope (IX70, Olympus, Japan) with a x60 objective lens was used in the system, and video images of beating cardiac myocytes were captured by a CCD camera in 60 fps (Figure 2(a)). A water bath was placed on the table of the microscope, and an ultrasound transducer was positioned so that its focal point was consistent with that of the objective lens (Figure 2(b)). A hole of about 10 mm in diameter was made in the bottom plate of the water bath, and the bottom plate was sandwiched between two plates of cover glass. This hole was used for an observation chamber and filled with a microbubble suspension. The cover glass with cardiac myocytes was used for the upper plate of the chamber and placed upside down so that the microbubbles could make contact with the cell. Microbubbles with plastic shells (3—5 µm in diameter) were used in this experiment. The number of bubbles was 1 or 2 per 10 myocytes.

In the experiments, cardiac myocytes were exposed to pulsed ultrasound in the presence and absence of microbubbles.

![Figure 1](image1.png)

**Figure 1.** Incubated cardiac myocytes isolated from a neonatal rat. (a) Two days after start of incubation. (b) Six days after start of incubation.

![Figure 2](image2.png)

**Figure 2.** Observation system. (a) Total system. (b) Observation chamber.
absence of microbubbles. The center frequency of the pulsed ultrasound was 1 MHz, and the pressure amplitudes (peak-negative pressures) were set at 5 steps: —0.28, —0.55, —0.73, —0.92 and —1.1 MPa. Five pulses with these five pressures were irradiated to a cardiac myocyte sample in this order and threshold pressure to produce a PVC was recorded. Eleven samples were used for the experiments of ultrasound exposure in the presence of microbubbles and eight samples were used in the absence of microbubbles. Since pressure threshold of PVC production also depends on the dose of UCA [1,2], the numbers of bubbles used in ultrasound exposure experiments should be the same. We therefore used plastic-shelled microbubbles that do not rupture up to pressure amplitude of —0.92 or —1.1 MPa. We tried to release ultrasound pulses simultaneously with myocyte contraction; however, the release timing was adjusted manually and was not accurate.

3. Results and discussion

In the first experiment, 11 samples were exposed to ultrasound pulses in the absence of microbubbles. Although no damage was evident in the myocytes, extra contractions were observed simultaneously in all cells, synchronized with the ultrasound exposure under the condition of high pressure amplitude. Figure 3 (black bar) is a histogram of pressure threshold to produce PVCs in the absence of microbubbles. The X-axis is threshold pressure and the Y-axis is relative frequency of PVC production. In the case without microbubbles, PVC was observed in only one sample at —0.73 MPa and was observed at a rate of 45% at the maximum pressure amplitude of —1.1 MPa.

In the second experiment, eight samples of cardiac myocytes were exposed to ultrasound in the presence of microbubbles. In this experiment, extra contraction synchronized with ultrasound exposure was observed even at low-pressure amplitudes. At high amplitudes, cyclic contraction was stopped simultaneously with ultrasound exposure, and gradually recovered to the regular beating. After cessation of beating for about 10 seconds, pulsation resumed locally and propagated to all myocytes, resulting in synchronized beating. At these amplitudes, cell-membrane rupture was sometimes observed at the location of a bubble. Figure 3 (white bar) shows dependence of relative frequency of PVC production on ultrasound pressure. Production of PVC strongly depended on the pressure amplitude of ultrasound and the presence of microbubbles. Disturbance of contraction rhythm was observed even at the lowest pressure amplitude, —0.28 MPa, and relative frequency exceeded 60% at

![Figure 3. A histogram of pressure threshold to produce PVCs in the presence and absence of microbubbles.](image)
the second-lowest amplitude step of —0.55 MPa. In this histogram, only frequency of PVC occurrence was taken into account, although the extent of PVC production and the rate of disruption also depend on the parameters mentioned above. From these results, it was concluded that presence of microbubbles attached to a cell reduces the threshold of pulsed ultrasound exposure that causes temporary disturbance of cardiac myocyte pulsation.

Despite the limited number of bubbles compared to that of myocytes (10% of myocytes), all of the myocytes stopped cyclic contraction simultaneously. This means that a myocyte stimulated by a microbubble may send a signal to surrounding myocytes. Further studies to evaluate cell damage and to identify the signal transmission path are needed. In an in vivo situation, there is a capillary wall between a myocyte and a microbubble, whereas a microbubble attached directly to a myocyte in our experiment. Therefore, the absolute value of pressure threshold might be higher than that obtained in present study.

4. Conclusions
An in vitro experimental method for PVC production was proposed to study the side effects of echo contrast imaging. Cardiac myocytes isolated from neonatal rats were used to directly observe changes in cardiac myocytes. Using this method, threshold pressure amplitudes to produce PVCs in the presence and absence of microbubbles were estimated. It was clearly shown that presence of microbubbles attached to a cell reduces threshold pressure for producing PVCs, and it was concluded that the proposed method is useful for studying the mechanism of PVC production.

5. References