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NOTE

Physiology

Reciprocal ST segment changes reproduced in burn-induced subepicardial injury model in bullfrog heart

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ABSTRACT. In our previous studies, by simply inducing burn injuries on bullfrog hearts or partially exposing their surface to high-potassium (K^+) solution, we could reproduce a ST segment elevation in the electrocardiogram (ECG), which is a characteristic finding in human ischemic heart disease. In the present study, using our burn-induced subepicardial injury model, we could additionally reproduce "reciprocal" ST segment changes for the first time in frog hearts, mimicking those observed in human acute myocardial infarction. Immunohistochemistry demonstrated markedly decreased Na^+/K^+-ATPase protein expression in the ventricular surface after the burn injury. The loss of this pump expression in injured cardiomyocytes was thought to be responsible for the creation of "currents of injury" and the subsequent ST segment changes observed in acute myocardial infarction.

KEY WORDS: acute myocardial infarction, bullfrog heart, Na^+/K^+-ATPase expression, reciprocal ST segment change

Acute coronary syndrome (ACS, formerly called "ischemic heart disease"), including acute myocardial infarction and unstable angina pectoris, is one of the leading causes of death worldwide [1, 12, 14]. To rapidly diagnose acute myocardial infarction and improve the outcome, the electrocardiogram (ECG) is the most useful test in patients with possible myocardial ischemia [16]. However, not all patients with acute myocardial infarction present typical ECG findings represented by an elevation of the ST segment, which is the interval between the ventricular depolarization and repolarization [13]. Additionally, an elevation of this segment is also observed in other normal or abnormal cardiac conditions, such as early repolarization, right bundle-branch block, left ventricular hypertrophy or pericarditis [9]. "Reciprocal" ST segment change, which is frequently observed in acute anterior or inferior myocardial infarction, is defined as an ST segment depression in leads opposite to those that reflect an ST segment elevation [17]. Since reciprocal ST segment change is specifically noted in acute myocardial infarction, the presence of this change strongly supports its diagnosis [2, 18] and also reflects the extent of myocardial ischemia [6]. In our previous studies, by simply inducing burn injuries on the bullfrog heart or partially exposing the heart surface to high-potassium (K^+) solution, we were able to reproduce an ST segment elevation in the ECG, which is a characteristic finding in human ischemic heart disease [7, 11]. Here, using our burn-induced subepicardial injury model, we could reproduce reciprocal ST segment changes in frog hearts for the first time that mimicked those observed in human acute myocardial infarction. By immunohistochemistry, we additionally examined the Na^+/K^+-ATPase protein expression in burned heart ventricle and revealed the physiological mechanisms underlying the ST segment changes in ECG.

Adult male bullfrogs, weighing 450 to 550 g (n=12), were purchased from Ohuchi Shōten (Saitama, Japan). After initial inhalation with isoflurane (Pfizer Inc., New York, NY, USA), the frogs were subjected to intramuscular injection of a long-acting anesthetic, ethyl carbamate (0.50 g/kg; Wako Pure Chemical Industries, Ltd., Osaka, Japan) as described previously [7, 8, 11]. Under deep anesthesia, the frog heart was surgically exposed and the electrical signals were directly recorded using an ECG electrode connected to an amplifier [7, 8, 11]. ECG waveforms were monitored and recorded in a data logger (midi LOGGER HV GL2000, GRAPHTEC Corp., Yokohama, Japan). All experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Miyagi University. To induce subepicardial injury in some frog heart ventricles (n=6), we heated the tip of a glass capillary tube with a diameter of 1.5 mm in a flame to more than 600°C and immediately placed it onto the ventricular surface (Fig. 1A). By applying the heated tube several times, we made some overlapping burn injuries in the subepicardial myocardium contiguous to the ventricular surface where the ECG recording electrode was set (Fig. 1A). In the other

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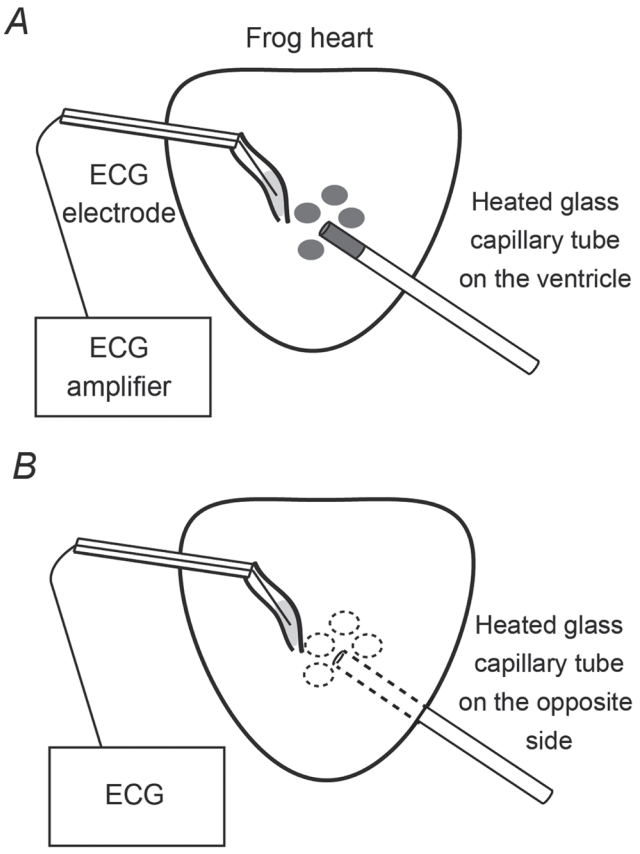

Fig. 1. Induction of subepicardial burn injury in bullfrog heart. (*A*) To induce subepicardial burn injuries in some frog hearts (n=6), a heated glass capillary tube was repeatedly placed on the ventricular wall adjacent to the ECG recording. (*B*) In the other frog hearts (*n*=6), burn injuries were similarly induced on the side opposite to the ventricle where the electrocardiogram (ECG) electrode was placed.

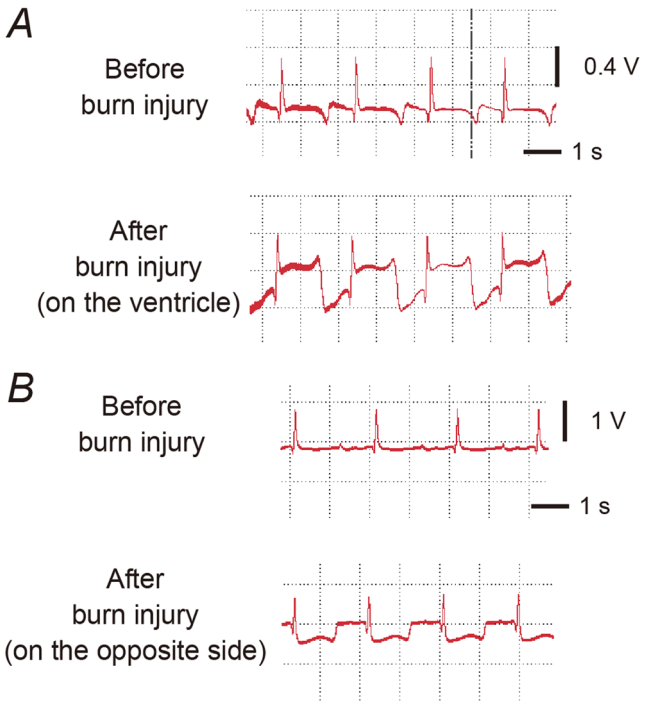

Fig. 2. Changes in electrocardiogram (ECG) before and after burn injury. (*A*) ECG changes before (*top*) and after burn injuries were induced on the ventricular surface (*bottom*). (*B*) ECG changes before (*top*) and after burn injuries were induced on the opposite side of the ventricle (*bottom*).

frog hearts (*n*=6), burn injuries were similarly induced on the side opposite to the ventricular surface where the ECG electrode was placed (Fig. 1B).

Consistent with our previous findings [11], the normal ECG showed a series of QRS complexes and the following T waves were made before the burn injuries (Fig. 2A top). As shown in Fig. 2A, the ST segments recorded between these waves were on the isoelectric line. Then, quickly after burn injuries were induced on the ventricular surface (Fig. 1A), the ECG showed a marked elevation of the ST segment from the isoelectric line (567 ± 101 mV, *n*=6; Fig. 2A bottom), indicating that myocardial injury had occurred. In contrast, when burn injuries were induced on the opposite side of the ventricular surface (Fig. 1B), the ECG demonstrated a marked depression of the ST segment from the isoelectric line (476 ± 53.7 mV, *n*=7; Fig. 2B bottom). The morphological patterns of the depressed ST segments were symmetrical to those of the elevated ST segments (Fig. 2B bottom vs. Fig. 2A bottom). This mimicked the "reciprocal" ST segment changes frequently observed in human acute myocardial infarction [17], indicating the presence of myocardial injury on the side opposite to the ventricle where the ECG electrode was placed.

The mechanism, by which such reciprocal ST segment changes were observed, could primarily be explained by the "currents of injury" as follows. The currents of injury are usually created from the injured subepicardium and flow towards the normal ventricular surface [10]. When myocardial injury was induced on the same side of the ventricular surface where the ECG recording electrode was placed (Fig. 1A), the currents of injury flow away from the electrode (Fig. 3Aa). Since the currents flow during the diastolic phase of the cardiac cycle, the ECG vector during this phase was negatively deflected from the isoelectric line (Fig. 3Ab), which allowed the ST segment to appear elevated during the systolic phase. In contrast, when myocardial injury was induced on the opposite side of the ventricular surface where the ECG electrode was placed (Fig. 1B), the currents of injury flow towards the electrode (Fig. 3Ba) in the direction opposite to the currents shown in Fig. 3Aa. This caused the ECG vector to be positively deflected during the diastolic phase, allowing the ST segment to appear depressed during the systolic phase (Fig. 3Bb). In the present study, using frog hearts, we were able to induce reciprocal ST segment changes for the first time in a burn-induced subepicardial injury model. This frog heart model appears to be suitable for demonstrating the mechanisms of such ECG changes.

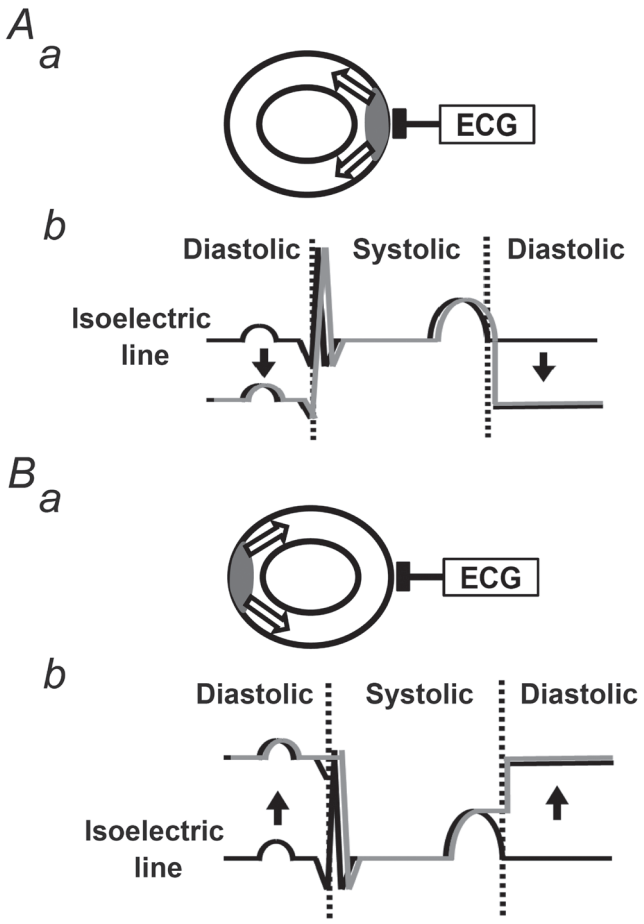

Fig. 3. Mechanisms of reciprocal ST segment changes in subepicardial burn injury model. (*A*) Mechanisms of ST segment elevation. When myocardial injury was induced on the same side of the ventricular surface where the ECG recording electrode was placed (*a*), the "currents of injury" (white arrows) that arose from the damaged subepicardium flowed away from the electrode. Therefore, the electrocardiogram (ECG) vector during the diastolic phase showed a negative deflection from the isoelectric line (*b,* arrows), making the ST segment appear elevated during the systolic phase (gray waveform). (*B*) Mechanisms of ST segment depression. When myocardial injury was induced on the opposite side of the ventricle where the ECG recording electrode was placed (*a*), the "currents of injury" (white arrows) flowed towards the electrode. In such cases, the ECG vector during the diastolic phase showed a positive deflection from the isoelectric line (*b,* arrows), making the ST segment appear depressed during the systolic phase (gray waveform).

In frog hearts, on the surface of the ventricle beneath the pericardial cavity, there are layers of cardiac muscles (Fig. 4A, left). Using Masson's trichrome staining, the cardiac muscles were shown to be covered by the epicardium (visceral pericardium), which is comprised of a single monolayer of mesothelial cells and loose connective tissue (Fig. 4B, left). After inducing burn injuries in the frog heart ventricle, a number of inflammatory cells infiltrated into the epicardium and most of the cardiac muscles became atrophic (Fig. 4A, right). The connective tissue layer within the epicardium became thicker with fibrous materials and the atrophic muscles were fibrously degenerated (Fig. 4B, right). Na^+/K^+-ATPase is an ion pump that normally transports sodium (Na^+) ions out of the cell and K^+ ions into the cell [5]. In frog heart ventricle, consistent with previous findings [11, 19], immunohistochemistry for Na^+/K^+-ATPase α-subunit (1:50; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) demonstrated its ubiquitous expression on the plasma membrane throughout the cardiomyocyte (Fig. 4C left). In our recent study, the pharmacological and functional blockade of this pump activity was deeply associated with the ST segment elevation in the ECG [11], as it generated the K^+ concentration gradient across the plasma membrane. In the present study, since ST segments were similarly elevated or reciprocally depressed after burn injuries (Fig. 2), we examined the protein expression of Na^+/K^+-ATPase in the burned heart ventricle (Fig. 4C, right). In the atrophic and fibrously degenerated cardiac muscle fibers on the ventricular surface, immunohistochemistry for Na^+/K^+-ATPase demonstrated the almost total absence of this protein expression (Fig. 4C, right).

In human ischemic heart diseases, including angina pectoris and acute myocardial infarction, hypoxic cardiomyocytes deplete the cytosolic adenosine triphosphate (ATP) concentration. This functionally inhibits the activity of the Na^+/K^+-ATPase, which transports the ions ATP-dependently [4, 5]. Therefore, such "functional" blockade of this pump activity has been considered to be primarily responsible for the pathology of ischemic heart disease [11]. In our burn injury model, as previously demonstrated in patients with early stage heart failure [15], the degeneration of cardiac muscles almost completely diminished the protein expression of Na^+/K^+-ATPase (Fig. 4C), resulting in the "expressional" blockade of this pump. Thus, in injured cardiomyocytes, K^+ ions were restrained from being transported into the cells, causing a decrease in their cytosolic concentration but an increase in their extracellular concentration. Based on the Nernst equation [3], this generates a significant difference in the resting membrane potential between the normal and injured cardiac muscles. As we recently demonstrated in frog hearts exposed to high K^+ solution [11], such an electrical difference in cardiomyocytes would create the "currents of injury", causing the ST segment changes shown in Fig. 3.

In conclusion, using a burn-induced subepicardial injury model in frog hearts, we were able to reproduce reciprocal ST segment changes for the first time, mimicking those observed in human acute myocardial infarction. The decreased Na^+/K^+-ATPase

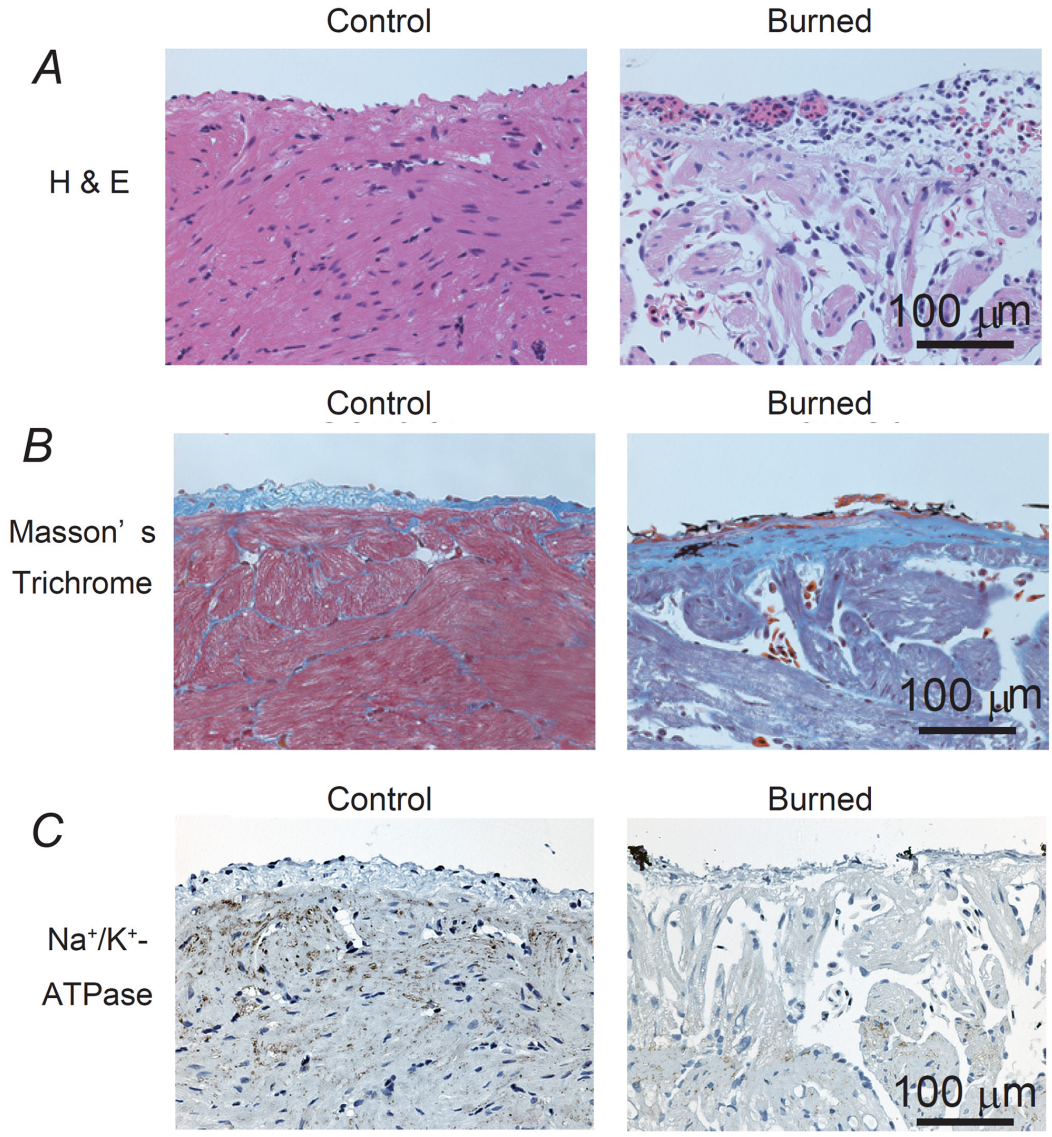

Fig. 4. Morphological changes in ventricular surface and Na^+/K^+-ATPase expression after burn injury in bullfrog heart. Hematoxylin and eosin (H&E) (*A*) and Masson's trichrome (*B*) staining in intact ventricular cardiomyocytes (Control) and those after the burn injury (Burned). Magnification ×20. (*C*) Immunohistochemistry using an antibody for Na^+/K^+-ATPase α-1 subunit (brown), counterstained with hematoxylin in intact ventricular cardiomyocytes (Control) and those after the burn injury (Burned). Magnification ×20.

expression in injured cardiomyocytes was thought to be responsible for the creation of "currents of injury" and the subsequent ST segment changes observed in acute myocardial infarction.

CONFLICT OF INTEREST. None declared.

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