EFFECTS OF DIFFERENT COMPONENTS OF SERUM AFTER BURN ON THE L-TYPE CALCIUM CHANNEL OF CULTURED MYOCARDIAL CELLS

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SUMMARY. *Objective:* The objective of the study was to observe the effects of different components of serum after burn on the L-type calcium channel of cultured myocardial cells. *Method:* Different components of serum were separated from the rat 6 h post-burn. The myocardial cells were separated from healthy rat and cultured *in vitro*, and the activity of the L-type channel was recorded by the patch-clamp technique. *Results:* After burn, the serum components enhanced the open action of the L-type calcium channel of cultured myocardial cells by increasing open-state probability, prolonging open time, shortening closed time, and enhancing current amplitude. Among the components, the low molecule (< 8 kD) fraction and lipid component were more effective. The effects of the lipid component could be suppressed by superoxide dismutase. *Conclusion:* After burn, some serum components could influence the activity of the L-type calcium channel, which might be an important cause of the dysfunction in cardiovascular system after burn.

Introduction

It has been confirmed that the cardiac systolic and diastolic functions are depressed *in vivo*[1] and *in vitro*[2,3] soon after burn trauma. Also, the components of blood serum may change and some abnormal substances may appear post-burn.[4,5] Furthermore, it has been found that the newly produced substances in post-burn serum may suppress the cardiac function of normal rats *in vitro*.[6,7] This indicates that some components of blood serum produced after burn may be partly responsible for the post-burn depression of cardiac function. It has also been reported that calcium metabolism was changed post-burn.[8,9] Also, the functional defect of cardiac contractility was paralleled by a significant rise in $Ca^{2+}i$ of the myocardium cells. Verapamil, a calcium channel blocker, was beneficial for cardiac function post-burn.[1] This indicates that the inhibition of cardiac contractility caused by burn trauma is correlated with the overload of cytosolic free calcium in the heart.[8,9] In order to further explore the mechanism of the influence of different components of serum on cardiac function, a study was made of the effects of different components of serum after burn on the L-type calcium channel of cultured myocardial cells.

Materials and methods

Ventricular myocytes were enzymatically dissociated from neonatal rat born 20 to 48 h previously, according to the method of Boerma,[10] and incubated in Dulbecco's modified Eagle's medium for 2 to 5 days before use.

Adult Wistar rats (170-240 g) received either a burn trauma (30% total body surface area) or a sham burn, as previously described.[1] Six hours later, the sera were collected from the rats and separated by dialysis and rotating distillation into the following three components: high molecular components (> 8-10 kD), low molecular components (< 8-10 kD), and lipid components.

The standard configuration for the single-channel tight seal patch-clamp technique[11] was used to record channel currents from the cultured cardiac myocytes. The patch pipettes were made from borosilicate glass capillary tubules by means of a two-stage patch pipette puller (PP-83, Narishige Co. Ltd, Tokyo, Japan) and fire-polished. The cells were superfused with a bath solution that contained (in mM) 140 KCl, 10 EGTA, 10 HEPES, 10 glucose, adjusted to pH 7.4 with KOH. The pipettes were filled with a solution containing (in mM) 110 $BaCl_2$, 10 HEPES, 30 choline Cl, 0.003 TTX, adjusted to pH 7.4 with KOH. The open-tip pipette resistance was 3-8 Mø when placed in the solution. A hydraulic micromanipulator (Narishige) was used to guide the patch microelectrode to the cultured myocardiac cells. High-resistance giga-ohm seals (up to 50 Gø) were obtained between the tip of the pipette and the cell membrane by applying gentle suction to

the pipette just after it touched the cell membrane. The bath Ag-AgCl ground electrode was connected to the control KCl bath through a 3% agar-KCl bridge. Unitary currents were recorded from membrane patches in the cell-attached configuration. Current signals were amplified by a patch-clamp amplifier (CEZ-2300, Nihon Kohden, Japan) and stored in a computer. Pulse generation, data acquisition, and analysis of the signals were performed using pCLAMP software (version 6.0.2, Axon Instruments Inc.). The capacitive artefact was minimized by the use of a low level of solution in the pipette and by coating the electrodes with N,N-dimethyltrimethylsilylamine (Fluka Chenie, Switzerland). Unitary currents were evoked by a 300 ms +60 mV depolarizing pulse from a holding potential of -50 mV. All the experiments were performed at a room temperature of 20-22 °C. Results are presented as mean ± SEM. Student's t test for paired data was used to compare the components of serum from the burn with the components of serum from the sham burn. A value of $p < 0.05$ was considered statistically significant.

Results

Effects of serum from burned rats on the L-type calcium channel of normal cardiac myocytes. The currents of the L-type calcium channel were not influenced by the serum from sham burned rats at the concentration of 20 Ìl serum per ml of bath solution used in this experiment, but were stimulated by the serum from burns at the same concentration and had an average unitary conductance increased from 1.125 ± 0.082 pS to 3.601 ± 0.112 pS (n = 5). In addition, the open time was prolonged, the closed was time shortened, and the open-state probability increased significantly.

Effects of the high molecular components of serum (> 8-10 kD) from burned rats on the L-type calcium channel of normal cardiac myocytes. After addition of the high molecular components (> 8-10 kD) of serum from sham burn and serum from burn to the bath solution, respectively, with a final concentration equal to 20 Ìl serum per ml of bath solution, there was no significant change in the activity of the L-type calcium channel. When the final concentration was increased up to 5 times, the open action of the L-type calcium channel was slightly enhanced, but there was no significant difference between serum from sham burn and that from real burn.

Effects of the low molecular components of serum (< 8-10 kD) from burned rats on the L-type calcium channel of normal cardiac myocytes. The low molecular components (< 8-10 kD) of serum from sham burn did not significantly influence the open action of L-type calcium at the final concentration of 20 Ìl serum per ml of bath solution, while the low molecular components of serum from burn significantly enhanced the open action of L-type calcium at the same concentration by increasing open-state probability, enhancing current amplitude, prolonging open time, and shortening closed time. Also, the fit statistics model of open duration distributions was changed from first order exponential-fitting method to second order exponential-fitting method. The effects of the

Table I - Effects of different component of serums on the L-type Ca2+ channel of myocytes (± s, n = 5)

Group	I (pA)	Open dwell time (ms) Ù1	Ù2	Closed dwell time (ms) Ù1	Ù2	pO
N	1.125 ± 0.082	0.087 ± 0.009		0.485 ± 0.046	12.913 ± 1.232	0.016 ± 0.001
S1	1.165 ± 0.075	0.085 ± 0.011		0.480 ± 0.031	15.002 ± 1.258	0.015 ± 0.001
B1	3.601 ± 0.112*	0.453 ± 0.044*		0.450 ± 0.033	3.399 ± 0.202*	0.097 ± 0.005*
S2	1.138 ± 0.085	0.089 ± 0.012		0.485 ± 0.039	13.015 ± 1.375	0.017 ± 0.001
B2	1.130 ± 0.078	0.092 ± 0.009		0.490 ± 0.042	14.050 ± 1.287	0.019 ± 0.002
S3	1.255 ± 0.091	0.072 ± 0.010		0.474 ± 0.040	14.990 ± 1.300	0.018 ± 0.001
B3	1.804 ± 0.102*	0.043 ± 0.008*	0.321 ± 0.020*	0.103 ± 0.009*	2.382 ± 0.202*	0.030 ± 0.002*
S4	1.090 ± 0.080	0.080 ± 0.005		0.453 ± 0.038	13.211 ± 1.300	0.018 ± 0.001
N + SOD	1.066 ± 0.079	0.088 ± 0.006		0.476 ± 0.040	14.232 ± 1.355	0.018 ± 0.002
B4	3.974 ± 0.203*	0.057 ± 0.006*	0.470 ± 0.027*	0.094 ± 0.006*	2.186 ± 0.180*	0.086 ± 0.006*
B4 + SOD	1.227 ± 0.082#	0.040 ± 0.004*#	0.354 ± 0.021*#	0.103 ± 0.009*	4.622 ± 0.206&#	0.038 ± 0.004*#

pA: pico-ampères; ms: microseconds: pO: probability of channel being open; N: normal control group; S1: effects of serum from sham burn; B1: effects of serum from burn; S2: effects of high molecular components (> 8-10 kD) of serum from sham burn; B2: effects of high molecular components of serum from burn; S3: effects of low molecular components (< 8-10 kD) of serum from sham burn; B3: effects of low molecular components of serum from burn; S4: effects of lipid components of serum from sham burn; B4: effects of lipid components of serum from burn; N + SOD: effects of superoxide dismutase on normal control group; B4 + SOD: effects of superoxide dismutase on B4 group; * = $p < 0.05$ vs control group; # = $p < 0.05$ vs

low molecular components of serum from burn on the L-type calcium channels were reversed after the low molecular components of serum were washed out.

Effects of the lipid components of serum from burned rats on the L-type calcium channel of normal cardiac myocytes. The lipid components of serum from sham burn did not significantly influence the activity of L-type calcium at the concentration of 0.1 mg/ml, while the lipid components of serum from burn significantly enhanced the open action of L-type calcium at the same concentration, which was similar to the effects of the low molecular components of serum in the burn group. Also, the effects of the lipid components of serum from burn on the L-type calcium channel could be weakened by 1000 U/ml superoxide dismutase (SOD), while SOD itself had no effect on the L-type calcium channel of normal cardiac myocytes at a concentration of 1000 U/ml and 3000 U/ml.

Discussion

Calcium channels play a vital role in several functions of the cardiovascular system. They carry a significant amount of depolarizing current necessary for the propagation of cardiac action potentials, play a major role in excitation-contraction coupling, and are sensitive to modulation by neurotransmitters.[12]

Cutaneous burn trauma causes cardiac contraction and relaxation defects, but the mechanism is unclear. Xia et al.[9] reported that burn-related changes in myocyte handling of calcium may play an important role in post-burn cardiac dysfunction. With the use of a high dissociation constant (K(d)) calcium indicator, they examined the correlation between changes in cytosolic free calcium concentration and cardiac function after burn trauma over 40% of the total body surface area in Sprague-Dawley rats. They confirmed that burn trauma impaired cardiac contractility and that this functional defect was accompanied by a significant rise in Ca^{2+}i in the heart. We confirmed that Verapamil, a calcium channel blocker, could benefit post-burn cardiac function.[1] Other studies found that the components of blood serum might be changed and that some abnormal substances appeared post-burn,[4,5] which could suppress the cardiac function of normal rat heart *in vitro*.[6,7] These results prompted us to investigate whether the serum components post-burn affected the activity of the L-type calcium channel, which is a main way of altering Ca^{2+}i.

This paper shows that the serum collected from burned rat can stimulate the L-type calcium channel. The effects included increasing open-state probability, prolonging open time, shortening closed time, and enhancing current amplitude. On the basis of these results we hypothesized that the effects of the newly produced serum components after burn caused a rise in Ca^{2+}i in the heart *in vivo*, which could be involved in the induction of post-burn cardiac dysfunction owing to stimulation of the L-type calcium channel.

Among the components of serum from burned rats, the high molecular components (> 8-10 kD) did not have significant effects on the L-type calcium channel, while the low molecular components (< 8-10 kD) and the lipid components stimulated the L-type calcium channel. The effects of the lipid components in the burn group could be partly reversed by SOD (which is a kind of oxygen radical scavenger). This suggests that the newly produced lipid components of serum after burn are associated with oxygen radical damage in burn injury.[13]

As for the kinetic analysis, the L-type calcium channel exhibited conventional brief openings in the normal control group. The open-time histogram was fitted by one exponential component, while the closed-time histogram was fitted by two exponential components, conforming with the three-state model of one open state and two closed states.[14] Stimulated by the low molecular components (< 8-10 kD) and the lipid components of the serum of burned rats, the open-time histogram for the Ca^{2+} channel was fitted by two exponential components instead of

RESUME. *But*: Les Auteurs de cette étude se sont proposés d'observer les effets de divers composants du sérum après la brûlure sur le canal du calcium type L des cellules myocardiaques cultivées. *Méthode*: Des composants différents du sérum ont été séparés du rat à 6 h après la brûlure. Les cellules myocardiaques ont été séparées du rat sain et cultivées *in vitro*, et l'activité du canal de type L a été enregistrée moyennant la technique "patch-clamp". *Résultats*: Après la brûlure, les composants du sérum ont augmenté l'action ouverte du canal de calcium de type L des cellules myocardiaques cultivées en augmentant la probabilité de l'état ouvert, prolongeant le temps ouvert, raccourcissant le temps fermé et augmentant l'amplitude du courant. Entre les composants, la fraction basse de la molécule (< 8 kD) et le composant lipidique étaient plus efficaces. Il était possible de supprimer les effets du composant lipidique moyennant la dismutase superoxide. *Conclusion*: Après la brûlure, certains composants du sérum pourraient influencer l'activité du canal du calcium type L, qui pourrait être une cause importante du dysfonctionnement du systè-

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