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Enhancement of norepinephrine-induced transient contraction in aortic smooth muscle of diabetic mice.

Asaki Abe*

Chiaki Kawasoe†

Yasuhiro Kondo‡

Katsunori Sato**

*Okayama University,

†Okayama University,

‡Okayama University,

**Okayama University,

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Asaki Abe, Chiaki Kawasoe, Yasuhiro Kondo, and Katsunori Sato

Abstract

Changes in norepinephrine-induced transient contractions in Ca^{2+} -deficient solution were investigated in the aortic smooth muscles of diabetic ALS (alloxan-induced diabetes susceptible) mice. The transient contractions in diabetic mice were significantly larger than those in normal mice. The longer incubation of the muscle preparations in Ca^{2+} -deficient solution made the transient contractions smaller, probably due to the leakage and decrease in norepinephrine-releasable stored Ca^{2+} . The rate of this reduction in contraction was slower in diabetic mice. These results suggest that the leakage of intracellular stored Ca^{2+} caused by extracellular Ca^{2+} deficiency is attenuated in diabetic mice, contributing to enhanced norepinephrine-induced transient contractions.

KEYWORDS: diabetes mellitus, vascular smooth muscle, norepinephrine

*Short Communication***Enhancement of Norepinephrine-induced Transient Contraction in Aortic Smooth Muscle of Diabetic Mice**

Asaki Abe*, Chiaki Kawasoe, Yasuhiro Kondo, and Katsunori Sato

Department of Animal Science, Faculty of Agriculture, Okayama University, Okayama 700-8530, Japan

Changes in norepinephrine-induced transient contractions in Ca^{2+} -deficient solution were investigated in the aortic smooth muscles of diabetic ALS (alloxan-induced diabetes susceptible) mice. The transient contractions in diabetic mice were significantly larger than those in normal mice. The longer incubation of the muscle preparations in Ca^{2+} -deficient solution made the transient contractions smaller, probably due to the leakage and decrease in norepinephrine-releasable stored Ca^{2+} . The rate of this reduction in contraction was slower in diabetic mice. These results suggest that the leakage of intracellular stored Ca^{2+} caused by extracellular Ca^{2+} deficiency is attenuated in diabetic mice, contributing to enhanced norepinephrine-induced transient contractions.

Key words: diabetes mellitus, vascular smooth muscle, norepinephrine

Various changes in the vascular responsiveness induced by diabetes mellitus have been reported, including the enhancement and reduction of stimulant-induced contractions [1]. Previously we used newly-developed experimental diabetic animals, alloxan-induced diabetes susceptible (ALS) and resistant mice (ALR), to study the alterations in the functions of vascular smooth muscle and found that contractile responses to norepinephrine and prostaglandin $\text{F}_{2\alpha}$ were increased by a long-term diabetic state [2]. In the present study, we investigated the changes in norepinephrine-induced transient contractions of aortic smooth muscles of diabetic ALS mice in Ca^{2+} -deficient solution. It is believed that in Ca^{2+} -deficient solution norepinephrine causes transient contractions, mainly due to the release of intracellularly stored Ca^{2+} [3]. This stored Ca^{2+} , which is releasable through an inositol trisphosphate (IP_3)-operated channel, has a physiologically important role in norepinephrine-

induced contraction [4]. Therefore, investigations of the norepinephrine-induced transient contractions in Ca^{2+} -deficient solution are important in order to elucidate the mechanisms of diabetes-induced changes in the responses of vascular smooth muscles.

Methods

ALS mice were produced and maintained at Okayama University [5] and used in this study. Diabetes was induced by the injection of alloxan at a dose of 45 mg/kg into the caudal veins of 4 male ALS mice aged 11 weeks. Seven control mice were administered 0.9% NaCl solution. Blood and urine glucose levels were measured by a glucose oxidase method and a urine glucose test paper, respectively. At 4 months after the injection, the blood glucose levels of all of the alloxan-treated mice were apparently elevated to 420-480 mg/dl, while those of control mice remained at 110-130 mg/dl. Similarly, urine glucose was detected in alloxan-treated mice (3+, over 0.5% glucose) but not in control mice.

Animals were anesthetized by diethylether and killed

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*Corresponding author. Phone: +81-86-251-8335; Fax: +81-86-251-8388
E-mail: abeasaki@cc.okayama-u.ac.jp (A. Abe)

by exsanguination from the carotid artery. The thoracic aorta was isolated immediately and carefully, and placed in a physiological salt solution (PSS), which contained NaCl (136.9 mM), KCl (5.4 mM), CaCl_2 (1.5 mM), MgCl_2 (1.0 mM), NaHCO_3 (23.8 mM) and glucose (5.6 mM) saturated with a 95% O_2 -5% CO_2 gas mixture. The surrounding adipose and connective tissues and endothelium of the inner surface were removed. Then the aorta was cut into a helical strip approximately 1.5 mm wide. Four muscle preparations were obtained from each animal.

Each strip was put in a temperature-regulated bath (37 degrees C) containing 10 ml PSS and connected to a force-displacement transducer. Muscle tension was measured isometrically under a resting tension of 2 mN. Before the experiment, preparations were equilibrated for approximately 60 min, and contractions due to the application of high K^+ solution, which was made by substituting 60 mM NaCl with equimolar KCl, were repeated 4 times. At the end of each tension recording experiment, the wet weight of each preparation was measured.

The care of the animals and the experiments in this study were performed in accordance with the Guidelines for Animal Experiments of Okayama University Faculty of Agriculture.

The following drugs were purchased: alloxan monohydrate (Ishizu Seiyaku, Osaka, Japan), l-norepinephrine bitartrate (Wako Pure Chemical, Osaka, Japan), ethylene glycol bis(2-aminoethyl ether)-N,N,N',N'-2-tetraacetic acid (EGTA) (Sigma, St. Louis, MO, USA), Blood sugar test 124 028 (Boehringer-Mannheim, Mannheim, Germany) and Tes-tape (Shionogi, Osaka, Japan).

The results of the experiments are expressed using means and standard errors. Student's *t* test was applied to the statistical analysis, and the differences of the means were considered to be significant when the *P* value was less than 0.05.

Results

A high K^+ solution induced a sustained contraction in all of the muscle preparations. No difference in the magnitude of the high K^+ -induced contractions was shown between control and diabetic mice (3.04 ± 0.20 and 3.03 ± 0.17 N/g wet weight tissue, respectively). An application of 100 nM norepinephrine in PSS also induced a sustained contraction. The norepinephrine-induced sustained contraction in the diabetic mice was significantly larger

than in the controls (3.24 ± 0.13 and 2.50 ± 0.30 N/g wet weight tissue). When muscle preparations were treated with Ca^{2+} -removed and 0.5 mM EGTA-added PSS for 2, 5, 10 or 15 min, 100 nM norepinephrine induced a transient contraction in all of the muscle preparations of the diabetic and control mice aortae (Fig. 1). The sizes of the transient contractions are shown in Table 1. At each incubation time in the Ca^{2+} -deficient solution, the transient contractions in the diabetic mice were significantly larger than those in the control mice. These contractions became smaller as the muscle preparations were exposed to Ca^{2+} -deficient solution for longer periods, possibly because the amount of norepinephrine-releasable Ca^{2+} decreased. As previously reported [3, 6], the amount of releasable Ca^{2+} can be expressed relatively as $R/(R_t - R)$, where *R* is the magnitude of the norepinephrine-induced transient contraction, and R_t is the magnitude of the theoretically maximal contractile response, which is nearly equal to the magnitude of the norepinephrine-induced sustained contraction in the pres-

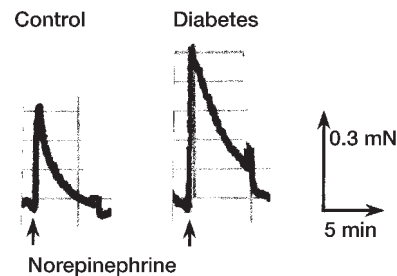


Fig. 1 Representative traces of norepinephrine-induced transient contractions of control and diabetic mouse aortae incubated in Ca^{2+} -deficient solution for 5 min.

Table 1 The magnitudes of norepinephrine-induced transient contractions in the aortic smooth muscle of control and diabetic ALS mice in Ca^{2+} -deficient solution

Time (min)	Control (mN/g)	Diabetes (mN/g)
2	450 ± 45	783 ± 61**
5	258 ± 20	479 ± 67**
10	95 ± 16	265 ± 20**
15	26 ± 4	165 ± 29**

Contractions (mN) were divided by the wet weight of each muscle preparation (g). Data are means and S.E.M. of 7 control and 4 diabetic mice. At each incubation time in Ca^{2+} -deficient solution, the contractions in the diabetic mice were significantly larger than in the control mice (**, *P* < 0.01).

ence of extracellular Ca^{2+} (Fig. 2). This formula is based on the supposition that R is proportional to the amount of the complex of Ca^{2+} that is liberated by norepinephrine and contractile protein in vascular smooth muscle cells. As shown in Fig. 3, $\log R/(R_t - R)$ was a linear function

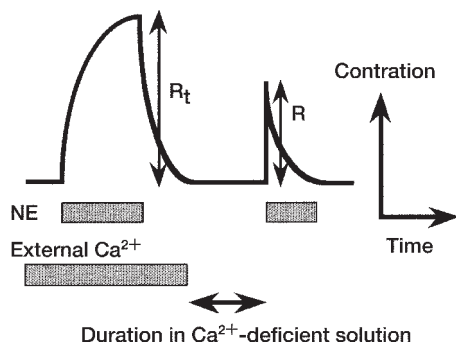


Fig. 2 Schematic illustration of contractions. Norepinephrine-induced sustained contractions were obtained in the presence of extracellular Ca^{2+} (normal PSS), and transient contractions were observed after various durations (2–15 min) of incubation in Ca^{2+} -deficient solution. The magnitudes of sustained (R_t) and transient (R) contractions were applied to calculate the amount of norepinephrine-releasable stored Ca^{2+} .

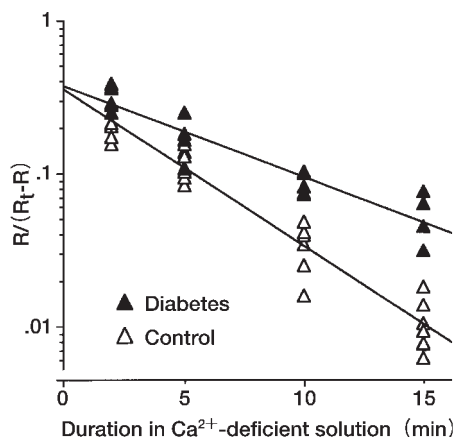


Fig. 3 Norepinephrine-induced transient contractions of control and diabetic ALS mouse aortae in Ca^{2+} -deficient solution. Ordinate: $\log R/(R_t - R)$, where R is the magnitude of the norepinephrine-induced transient contraction, and R_t is that of the theoretically maximal contractile response, which is nearly equal to the magnitude of the norepinephrine-induced sustained contraction in the presence of extracellular Ca^{2+} [3, 6]. The slope of the line indicates the rate of loss of Ca^{2+} from the intracellular storage site. Each symbol represents the datum for each animal. Open and closed triangles represent the control and diabetic ALS mice, respectively.

of the duration of the preceding Ca^{2+} -free incubation, and the amount of releasable Ca^{2+} was lost with a half-time of approximately 3 min in control mice and 5 min in diabetic mice.

Discussion

Investigators have reported under various experimental conditions that norepinephrine-induced sustained contraction of vascular smooth muscle was either increased [7], decreased [8] or unchanged [9] in diabetic animals. We previously reported that norepinephrine-induced contraction was potentiated when ALS mice were in a diabetic state for longer than 2 months [2]. It is thought that norepinephrine-induced contraction is at least partly due to the release of intracellular stored Ca^{2+} , and that norepinephrine-induced transient contraction in Ca^{2+} -deficient solution is mainly due to the release of stored Ca^{2+} . We studied the transient contraction in order to gain further understanding of the mechanism(s) of the alterations in norepinephrine-induced contractions that are caused by diabetes.

In the aortic smooth muscle of rats that received an injection of streptozotocin and fell into a diabetic state, the norepinephrine-induced transient contractions in Ca^{2+} -deficient solution were investigated. However, the results of these investigations were not consistent. Increases [7, 10] and decreases [11, 12] in the contractions were reported, and the reason for this discrepancy was not clear. In the present study, the norepinephrine-induced transient contractions of diabetic ALS mice aortae incubated in Ca^{2+} -deficient solution for 2–15 min were larger than those in normal ALS mice aortae. We already reported the enhancement of norepinephrine-induced sustained contractions in long-term diabetic ALS mice aortae in the presence of extracellular Ca^{2+} [2]. The enhancement of transient and sustained contractions in diabetic animals may be caused by common mechanisms, including the increase in the norepinephrine-triggered phosphoinositide metabolism that is seen in diabetes [13]. This mechanism should be specific to norepinephrine, for the response to 5-HT did not change under the same conditions [2].

We studied the effect of protracted incubation in a Ca^{2+} -deficient solution and made a new finding regarding the leakage of stored Ca^{2+} in diabetic animals. The longer incubation of vascular smooth muscle preparation under Ca^{2+} -deficiency made the norepinephrine-induced transient

contraction smaller, probably due to the leakage and the decrease of stored Ca^{2+} [3, 6]. The rates of these decreases of stored Ca^{2+} and transient contraction were slower in diabetic mice (Fig. 3). This finding reveals that the intracellular stored Ca^{2+} is lost with a longer half-time in diabetes. Therefore, long-term diabetes may attenuate the leakage of Ca^{2+} from intracellular storage sites in vascular smooth muscle cells and may preserve a relatively large amount of Ca^{2+} in the storage sites. This large Ca^{2+} release may at least partly explain the potentiation of the transient contraction and also the sustained contraction induced by norepinephrine in diabetic animals. Since diabetes is probably complicated by disorders of Ca homeostasis in the blood plasma [14], the reduction in peri-cellular Ca^{2+} and the attenuation of the leakage of stored Ca^{2+} may cause a disturbance in the regulation of intracellular Ca^{2+} movement, and may affect the norepinephrine-induced vascular responses *in vivo*. The reason that diabetes changes the leakage of Ca^{2+} remains unknown. It has been reported that the function and expression of the Na^+ - Ca^{2+} exchanger in the hearts of diabetic rats are diminished [15]. A similar change in vascular smooth muscle, if it exists, may reduce Ca^{2+} extrusion through cell membranes and result in the preservation of intracellular stored Ca^{2+} .

In conclusion, it was observed that long-term diabetes potentiates the norepinephrine-induced transient contraction in aortic smooth muscle of ALS mice in a Ca^{2+} -deficient solution. This effect may be caused by the attenuation of the leakage of Ca^{2+} from intracellular storage sites and by the preservation of a relatively large amount of norepinephrine-releasable Ca^{2+} .

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