Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 465 (2003) 153–161
www.elsevier.com/locate/ejphar

Caffeine-induced contracture in oesophageal striated muscle of normotensive and hypertensive rats

Fumiko Sekiguchi, Kyoko Kawata, Mayumi Komori, Satoru Sunano*

Department of Anatomy and Physiology, Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashiosaka, Osaka 577-8502 Japan

Received 10 December 2002; received in revised form 28 January 2003; accepted 4 February 2003

Abstract

To elucidate whether properties of the sarcoplasmic reticulum are altered, not only in vascular smooth muscle, but also in visceral striated muscle of spontaneously hypertensive rats (SHR), caffeine-induced contractures in oesophageal striated muscle of Wistar Kyoto rats (WKY) and stroke-prone SHR (SHRSP) were compared. In both preparations, 30 mM caffeine induced a contracture with two components. The second component, which was diminished by extracellular Ca^{2+} removal or Ni^{2+} but not by verapamil, was much smaller in SHRSP. Both components and differences between WKY and SHRSP coincided with changes in intracellular Ca^{2+}. Although membrane potential was identical between these preparations, caffeine induced slight depolarization only in WKY preparations. Similar depolarization was observed with 10 mM K^+, which induced no contraction. It is suggested that the first and the second components of caffeine-induced contracture were induced by Ca^{2+} released from sarcoplasmic reticulum and by Ca^{2+} that entered through channels activated by sarcoplasmic reticulum Ca^{2+} depletion, respectively. In SHRSP preparations, Ca^{2+} from the latter pathway was clearly decreased, although this change is thought not to be related to the initiation of hypertension. These results suggest that Ca^{2+} handling properties of cell membrane and sarcoplasmic reticulum are generally altered in muscles of SHRSP.

Keywords: Stroke-prone spontaneously hypertensive rat (SHRSP); Oesophageal striated muscle; Caffeine; Contracture; Ca^{2+} influx; Sarcoplasmic reticulum

1. Introduction

Changes in Ca^{2+} handling in contraction of vascular smooth muscles of spontaneously hypertensive rats (SHR) have been reported (Rousseau et al., 1988). These changes have been observed not only in vascular but also in visceral smooth muscles (Altman et al., 1976; Kwan et al., 1982). However, little is known about changes in excitation–contraction coupling (E–C coupling) of striated muscles of SHR, although changes in other properties of skeletal muscles have been reported (Atrakchi et al., 1994; Bachir-Lamrini et al., 1990; Carlsen and Gray, 1987; Carlsen et al., 1996a,b; Gray et al., 1994; Pickar et al., 1994; Syme et al., 1990). This may be because of a smaller or a lack of contribution of skeletal muscles to circulation, especially to the initiation of hypertension.

Among the changes in vascular smooth muscle, it has been reported that caffeine-induced contraction observed in the absence of extracellular Ca^{2+} was altered in preparations from SHR (Kanagy et al., 1994; Moriyama et al., 1989; Sugiyama et al., 1990). Sarcoplasmic reticulum plays more important roles in E–C coupling in striated muscle than in smooth muscle (see Endo, 1977; Fleischer and Inui, 1989; Sandow, 1965), and it has recently been reported that the response to caffeine is altered in the skeletal muscle of SHR when compared with that of normotensive Wistar Kyoto rats (WKY) (Bortolotto et al., 2001). It was of interest to investigate the changes in E–C coupling in visceral striated muscle, as various changes in visceral smooth muscle have been reported, as described above. The outer layer of the oesophagus is composed of striated muscle and, in this sense, is thought to be visceral striated muscle. We have recently observed marked differences between the actions of caffeine on the twitch contraction of this muscle from normotensive WKY and that from stroke-prone SHR (SHRSP) (Kawata et al., 2000; Sekiguchi et al., 2003). Based on this difference in the effect of caffeine, the

* Corresponding author. Tel.: +81-6-6721-2332, ext. 3827; fax: +81-6-6730-1394.
E-mail address: sunano@phar.kindai.ac.jp (S. Sunano).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01435-3

changes of sarcoplasmic reticulum properties in the oesophageal striated muscle of SHRSP were suggested. We now studied the contracture of the oesophageal striated muscle induced by a high concentration of caffeine to study further the changes in properties of sarcoplasmic reticulum in SHRSP, and found the effects markedly different from those on preparations from WKY.

2. Materials and methods

2.1. Animals and preparations

The rats were handled according to the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences" of the Physiological Society of Japan.

SHRSP and WKY at the age of 16 weeks were used in the present experiments. Systolic blood pressure was measured by the tail-cuff method. Prior to the measurement, the rats were warmed at 40 °C for 10 min to obtain constant and accurate blood pressure recording. These rats were killed by exsanguination from the vena cava under anesthesia with CO_2 gas, and the oesophagus was excised from the thoracic cavity. Strips of 2-mm width and 15-mm length were cut from the outer layer of the oesophagus in modified Tyrode's solution.

2.2. Solutions

A modified Tyrode's solution of the following composition was used (mM): NaCl, 137; KCl, 5.4; $CaCl_2$, 2.0; $MgCl_2$, 1.0; $NaHCO_3$, 11.9; NaH_2PO_4, 0.4; glucose, 5.6; equilibrated with a gas mixture of 95% O_2 and 5% CO_2 (pH at 37 °C was 7.3). In some experiments, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)-buffered solution was used. The HEPES-buffered solution was made by replacing $NaHCO_3$ and NaH_2PO_4 in the Tyrode's solution with 10 mM HEPES and was equilibrated with O_2 instead of 95% O_2 + 5% CO_2. K^+-Tyrode's solution was made by replacing all NaCl in the modified Tyrode's solution with KCl. Ca^{2+}-free Tyrode's solution was made by omitting $CaCl_2$ from the modified Tyrode's solution.

2.3. Measurement of contractures

Changes in force of the preparation were measured isometrically with a force–displacement transducer (Minevia, Nagano, Japan). After equilibration of the preparation in the modified Tyrode's solution for 60 min, the preparation was subjected twice to high-K^+-induced contractures by changing the solution from modified Tyrode's to high-K^+ Tyrode's solution for 3 min with an interval of 60 min. The effects of caffeine were examined after these procedures, so that consistent results could be obtained. In the present experiment, as the preparations from SHRSP were smaller than those from WKY, all developed tensions were normalized to a contracture induced by 142.4 mM K^+.

2.4. Measurement of intracellular Ca^{2+} level

The intracellular Ca^{2+} level was measured with the fluorescent Ca^{2+} indicator, fura-PE3. Briefly, the acetoxymethyl ester of fura-PE3 (fura-PE3/AM, 20 μM) was loaded into the outer layer strips of striated muscle of the oesophagus, 1-mm width and 7-mm length, in a dark room for 5 to 6 h at room temperature. Pluronic F-127 (0.06%) was added to increase the solubility of fura-PE3/AM. After loading, the preparations were washed with the modified Tyrode's solution at 37 °C for 15 min and mounted in a 5-ml CAF-110 chamber (Japan Spectroscopic, Tokyo, Japan) so that the changes in intracellular Ca^{2+} level and isometric contraction could be measured simultaneously. The temperature of the chamber was kept at 37 °C. The intracellular Ca^{2+} level was measured as the ratio of intensity of fluorescence at a wavelength of 500 nm with excitation at wavelength 340 and 380 nm applied alternately at a frequency of 128 Hz (F_{340}/F_{380}). The ratio (F_{340}/F_{380}) obtained in Ca^{2+}-free solution containing 20 mM EGTA was taken as 0% and that obtained in the presence of 142.4 mM K^+ was taken as 100%, and values obtained under other conditions were normalized using these two values.

2.5. Membrane potential

The membrane potential of oesophageal striated muscle was measured by the microelectrode technique with a glass microelectrode, which had a tip resistance of 40 to 60 MΩ. The preparations were placed horizontally in an organ bath with a volume of 1.5 ml and immersed under a constant flow (2.0 ml min^{-1}) of incubation medium at a constant temperature of 37 °C. The potential changes were amplified with a microelectrode amplifier (MEZ-8301, Nihon Kohden, Tokyo, Japan) and observed with a digital oscilloscope (COR 5521, Kikusui, Kawasaki, Japan). The data were stored at an acquisition rate of 100 Hz using PowerLab recording System (AD Instruments, Castle Hill, NSW, Australia) on a computer (ThinkPad i1400, IBM, USA). A sudden drop of the spot of the oscilloscope was regarded as penetration of the electrode through the cell membrane, and the value after the potential had reached a stable level was taken as the resting potential. The effects of caffeine were examined by adding the drug to the incubation medium.

2.6. Drugs

(*R*,*S*)-(3,4-Dihydro-6,7-dimethoxyisoquinoline-1-yl)-2-phenyl-*N*,*N*-di-[2-(2,3,4-trimethoxyphenyl)ethyl]-acetamide methane-sulfonic acid (LOE 908) was kindly provided by Boehringer Ingelheim Pharma (Ingelheim, Germany). Other drugs were obtained commercially from the following sources: caffeine, verapamil hydrochloride and nickel chlor-

ide were from Wako (Osaka, Japan), ryanodine and pluronic F-127 were from Sigma (St. Louis, MO, USA), ethylene glycol-bis(β-aminoethyl-ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) was from Dojindo (Kumamoto, Japan), 1-[β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1*H*-imidazole hydrochloride (SK&F 96365) was from Calbiochem (La Jolla, CA, USA) and fura-PE3/AM was from Texas Fluorescence Laboratory (Austin, TX, USA).

2.7. Statistics

The values obtained are expressed as means ± S.E.M. with the number of experiments given in parentheses. The differences in the values were analyzed by Student's unpaired *t*-test or by two-way analysis of variance (ANOVA) with Bonferroni/Dunn's post hoc test considering *P* values smaller than 0.05 to be significant.

3. Results

3.1. Body weight and blood pressure of rats

Body weights of WKY and SHPSP used in the present experiments were 367 ± 3.4 g ($n = 40$) and 270 ± 4.3 g ($n = 40$), respectively. Systolic blood pressures were 135 ± 1.0 mm Hg ($n = 40$) and 238 ± 1.7 mm Hg ($n = 40$), respectively, in WKY and SHRSP. The blood pressure of SHRSP was significantly higher than that of WKY ($P < 0.001$).

3.2. Caffeine-induced contracture of the preparations from WKY and SHRSP

In the oesophageal striated muscle from WKY, caffeine at concentrations between 1 and 3 mM induced a slow sustained elevation of basal force, which increased as the caffeine concentration was increased. Caffeine, 10 mM, induced a small rapid contractile response in addition to a slow increase of the basal force (data not shown). The two components could be distinguished most clearly when the contracture was initiated by 30 mM caffeine (Fig. 1A). At this concentration, the amplitudes of the first and second components were 80.1 ± 6.13% ($n = 6$) and 185.6 ± 17.89% ($n = 6$), respectively, of the amplitude of that induced by 142.4 mM K^+ (Fig. 1B).

In the preparation from SHRSP, the sustained increase of basal force was not observed at caffeine concentrations up to 3 mM. Caffeine at 10 mM induced only a small phasic contracture followed by a sustained but much lower increase of basal force (data not shown). At a caffeine concentration of 30 mM, the height of the first component of the contracture was 46.3 ± 5.58% ($n = 8$); this value was significantly smaller than that of the preparation from WKY. A more prominent difference was observed in the second component, which was only 8.0 ± 0.79% ($n = 8$) of that induced by 142.4 mM K^+ (Fig. 1).

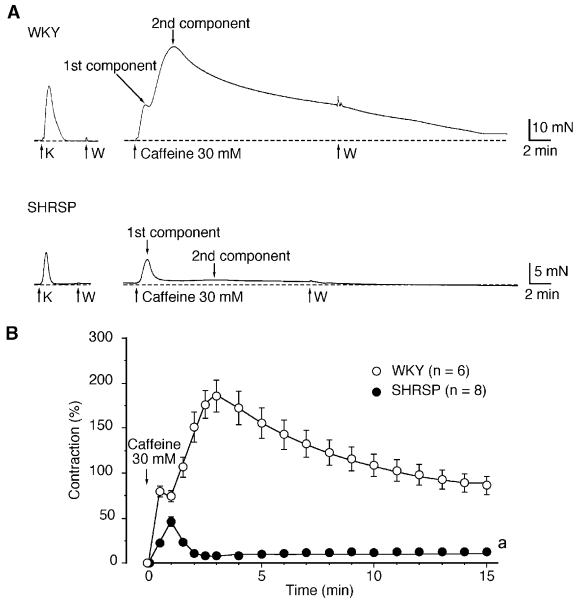

Fig. 1. Contractures induced by 30 mM caffeine in the outer layer of the striated muscle of the oesophagus of WKY and SHRSP. (A) Traces of caffeine-induced contracture in the preparations from WKY (upper) and SHRSP (lower). K and W indicate application of K-Tyrode's solution and washing out with normal Tyrode's solution, respectively. (B) Time course of caffeine (30 mM)-induced contracture in the preparations from WKY and SHRSP. [a]: $P < 0.001$, vs. the curve for the preparations from WKY.

When 100 mM caffeine (maximum solubility) was applied, the amplitude of the first component of the contracture increased in both preparations. The increase in the first component was more prominent in the preparations from SHRSP and the amplitude of the first component became similar in both preparations. However, the second component increased in the preparations from SHRSP, while it decreased in those from WKY. Thus, the contracture induced by 100 mM caffeine became similar (Fig. 2A).

Fig. 2B shows the contracture induced by cumulative application of caffeine in the preparations from WKY and SHRSP. Then concentration was increased cumulatively up to 100 mM, no further tension change was observed in the preparations from WKY. In the preparations from SHRSP, the increase in caffeine concentration up to 100 mM caused an increase in force, although the tension developed was much smaller than that observed on single application of 100 mM caffeine.

3.3. Effects of extracellular Ca^{2+} removal and Ca^{2+} influx blockers

The second component of the caffeine-induced contracture of both preparations was highly sensitive to extracellular Ca^{2+} and was almost abolished by removal of extracellular Ca^{2+} (in Ca^{2+}-free solution) even in the preparations from WKY, while the first component was

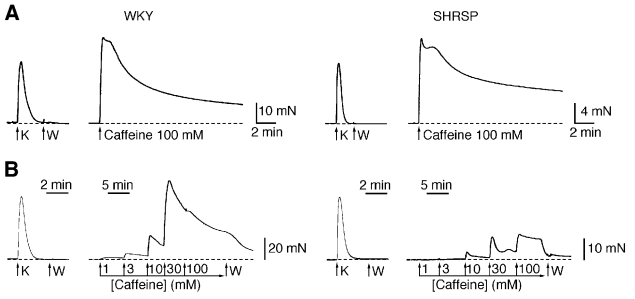

Fig. 2. Contractures induced by 100 mM caffeine and by cumulative application of caffeine in the preparations from WKY and SHRSP. (A) Contracture with 100 mM caffeine. (B) Contracture with cumulative application of caffeine. Others are the same as those in Fig. 1. Note the differences in configuration of contracture induced by 100 mM caffeine from those induced by 30 mM caffeine in Fig. 1A. Also, note that 100 mM caffeine did not induce a further increase in tension but rather accelerated relaxation in the preparations from WKY.

relatively insensitive to extracellular Ca^{2+} and was observed in Ca^{2+}-free solution without marked changes (Figs. 1A and 3A). Neither component of the caffeine-induced contracture of the preparations from WKY and SHRSP was affected by the application of 10^{-5} M verapamil, a voltage-sensitive Ca^{2+} channel blocker (Fig. 3B).

The caffeine-induced contracture of both preparations was not affected by LOE 908 (10^{-5} M), SK&F 96365 (10^{-5} M), both of which are known to block Ca^{2+} store-depletion-activated Ca^{2+} influx in certain cells (Encabo et al., 1996; Leung and Kwan, 1999) (data not shown). A high concentration of nickel ions (Ni^{2+}), which also has been reported to be a blocker of Ca^{2+} store-depletion-activated Ca^{2+} influx in certain cells (Kurebayashi and Ogawa, 2001), markedly depressed the second component of caffeine-induced contracture at 2×10^{-3} M, showing little effect on the first component in the preparations from WKY (Fig. 3C). The first component of the caffeine-induced contracture in the preparations from SHRSP was not influenced by Ni^{2+} (Fig. 3C).

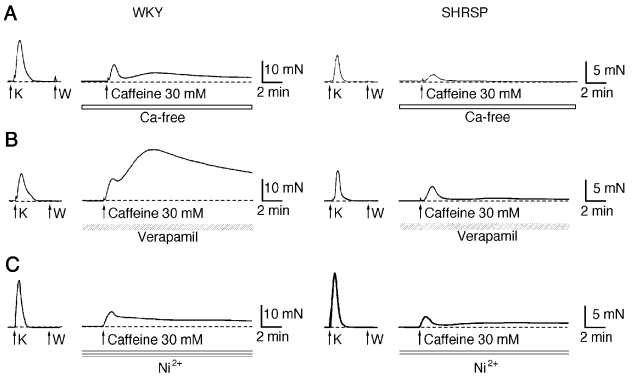

Fig. 3. Effects of extracellular Ca^{2+} removal, verapamil and Ni^{2+} on caffeine-induced contracture. (A) Caffeine-induced contracture in the absence of extracellular Ca^{2+}. (B) Caffeine-induced contracture in the presence of verapamil (10^{-5} M). (C) Caffeine-induced contracture in the presence of Ni^{2+} (2×10^{-3} M). Other points are the same as those in Fig. 1.

3.4. Effects of ryanodine

Ryanodine showed no obvious effect on caffeine-induced contracture in the preparations from WKY and SHRSP when applied prior to the application of caffeine. However, it prevented the loading of Ca^{2+} through soaking in normal Tyrode's solution after depletion of Ca^{2+} from sarcoplasmic reticulum, which was performed by application of caffeine in the absence of extracellular Ca^{2+}. Thus, no first component of contracture was observed on application of caffeine in the absence of extracellular Ca^{2+} (Fig. 4A and B). When the second application of caffeine was performed in the presence of extracellular Ca^{2+}, a second component of greater amplitude without the first component was observed (data not shown). Similarly, reintroduction of Ca^{2+} into Ca^{2+}-free solution in the presence of ryanodine and caffeine induced a marked contracture, which had a similar time course to the second component of caffeine-induced contracture (Fig. 4C). In the preparations from SHRSP, on the other hand, no or only weak slow contracture was initiated by the same procedures (Fig. 4D).

3.5. Intracellular Ca^{2+} level

Caffeine (30 mM) induced a rapid rise of intracellular Ca^{2+} level followed by a sustained elevation of the level as observed with the fura-2 method in the preparations from WKY (Fig. 5A, lower). The time course of the elevation of intracellular Ca^{2+} level corresponded with the first and the second components of contracture. In the preparations from SHRSP, the sustained phase of the elevation of Ca^{2+} level was markedly smaller than that of the preparations from WKY, although the first phase of the elevation was comparable in both preparations (Fig. 5B). Fig. 5C shows the relation between contraction amplitude and intracellular

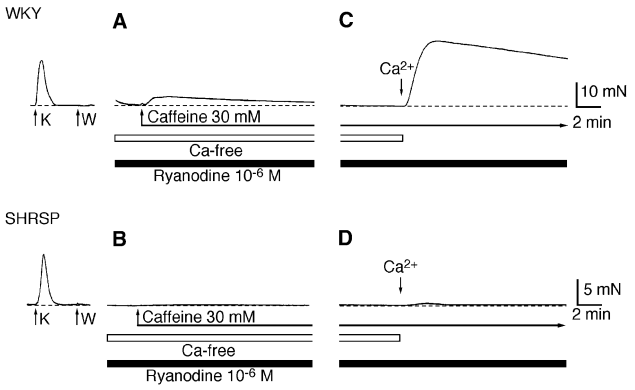

Fig. 4. Effects of ryanodine on caffeine-induced contracture in the absence of extracellular Ca^{2+} and reintroduction of Ca^{2+}. After initiation of contracture in the absence of extracellular Ca^{2+}, caffeine was washed out and Ca^{2+} was loaded by soaking in normal Tyrode's solution for 30 min in the presence of ryanodine (10^{-6} M). Then, caffeine was applied in the presence of ryanodine and absence of extracellular Ca^{2+} (A and B). C and D are continuous recordings of A and B, respectively; Ca^{2+} (2 mM) was reintroduced in the presence of caffeine and ryanodine.

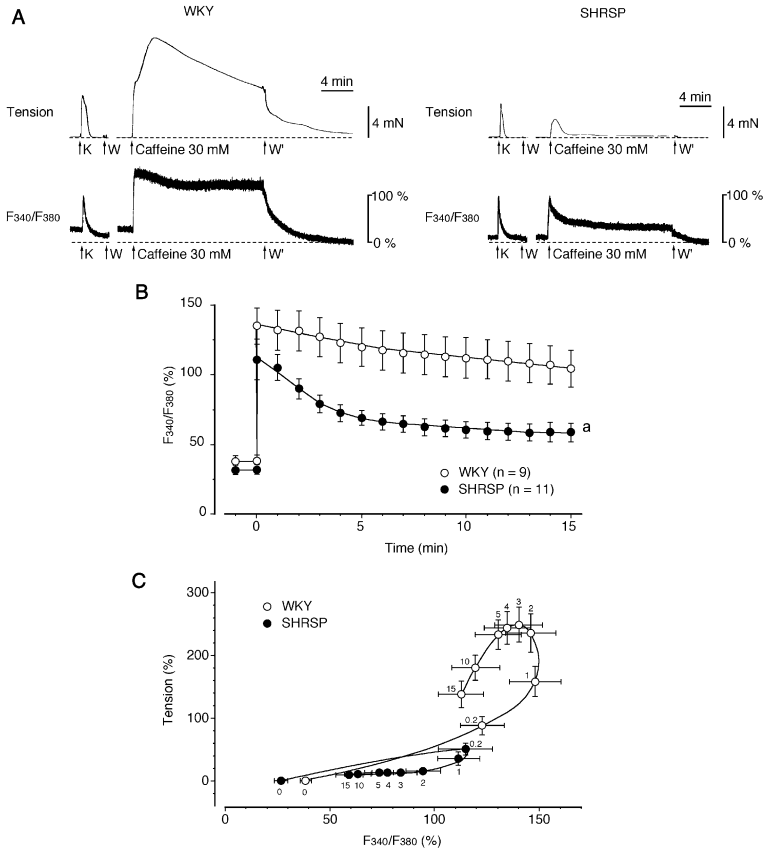

Fig. 5. Effects of caffeine on intracellular Ca^{2+} level in the oesophageal striated muscle from WKY and SHRSP. (A) Simultaneous recordings of changes in tension and intracellular Ca^{2+} level (F_{340}/F_{380}). W′: wash out with Ca^{2+}-free Tyrode's solution containing 20 mM EGTA. The reduced level of Ca^{2+} achieved by W′was taken as 0% and the maximum level observed with application of K-Tyrode's solution was taken as 100%, and changes in intracellular Ca^{2+} level are expressed as percentage of this value as described in Materials and methods. Other points are the same as those in Fig. 1. (B) Mean time courses of changes in intracellular Ca^{2+} level in the preparations from WKY and SHRSP. Caffeine was applied at time 0. (C) Time course of intracellular Ca^{2+} level (F_{340}/F_{380})-tension relation of 30 mM caffeine-induced contracture. Numerals beside symbols indicate the time after application of caffeine (min). [a]: $P<0.001$, vs. the curve of the preparations from WKY.

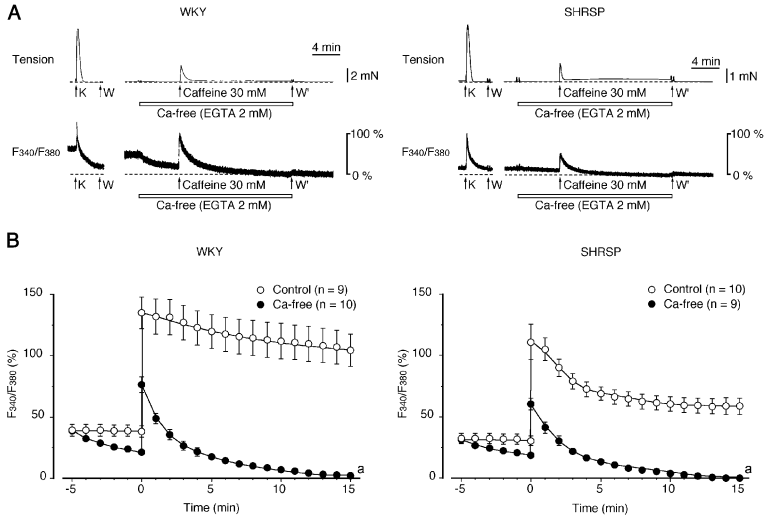

Fig. 6. Effects of removal of extracellular Ca^{2+} on the increase in intracellular Ca^{2+} level in the preparations from WKY and SHRSP. (A) Procedures were the same as those in Fig. 5 except for the removal of extracellular Ca^{2+} by applying Ca^{2+}-free solution containing 2 mM EGTA 5 min before application of caffeine. (B) Mean time course of the changes in intracellular Ca^{2+} level in the absence of extracellular Ca^{2+}. Extracellular Ca^{2+} was removed 5 min (-5) before application of caffeine and caffeine (30 mM) was applied at time 0. [a]: $P<0.001$, vs. the curve for control condition.

Table 1
Membrane potential of the oesophageal striated muscle in the resting state and in the presence of 30 mM caffeine

	Membrane potential (mV)			
	Resting	*n*	30 mM caffeine	*n*
WKY	−71.7 ± 0.59	33	−56.7 ± 1.16*	29
SHRSP	−72.5 ± 0.56	29	−72.9 ± 0.43**	27

* $P < 0.001$, vs. resting membrane potential.
** $P < 0.001$, vs. WKY data.

Ca^{2+} at various times after the application of caffeine. The relationship was hardly different between the preparations from WKY and SHRSP up to 0.2 min after the application of caffeine but was very after 1 min. From 1 to 3 min, the contraction height increased without change or a decrease in intracellular Ca^{2+} level in the preparations from WKY, while such changes were not observed in the preparations from SHRSP.

Caffeine induced only the first phase of the elevation of intracellular Ca^{2+} level in the absence of extracellular Ca^{2+} in the preparations from both WKY and SHRSP; the sustained elevation disappeared even in the preparations from WKY (Fig. 6).

3.6. Membrane potential

The resting membrane potential was not significantly different between preparations from WKY and SHRSP. In the presence of 30 mM caffeine, the membrane potential of the preparations from WKY was slightly but significantly depolarized, while that of the preparations from SHRSP was not significantly altered (Table 1).

Similar depolarization (about 15 mV) was observed when K^+ concentration of the modified Tyrode's solution was elevated to 10 mM. However, no change in muscle tension was observed in this case (data not shown).

4. Discussion

In skeletal muscles, caffeine has been shown to act directly on the ryanodine receptors of sarcoplasmic reticulum and release Ca^{2+}, which initiates contracture (see Endo, 1977; Rousseau et al., 1988; Ríos and Pizarro, 1991). Therefore, caffeine can also initiate contracture in skinned preparations of skeletal muscles (Duke and Steele, 1998; Endo et al., 1970; Lamb and Stephenson, 1990; Salviati and Volpe, 1988; Su, 1988). The contracture is transient and disappears within 1 min. In crayfish muscle, caffeine-induced contracture, which is also transient, has been reported to not be influenced by the removal of extracellular Ca^{2+} (Chiarandini et al., 1970). These results support the conclusion that caffeine-induced contracture is initiated by Ca^{2+} released from intracellular storage sites, i.e. sarcoplasmic reticulum, and that released Ca^{2+} is removed by mechanisms such as uptake via the Ca^{2+} pump of sarcoplasmic reticulum or extrusion through the cell membrane within a short period. The caffeine-induced contracture of the outer layer of the oesophagus studied in the present experiment may be initiated by mechanisms similar to those in other striated muscles, as this layer of the rat oesophagus is composed of striated muscle cells similar to rat skeletal muscle (Gruber, 1968). Then, the caffeine-induced contracture would be expected to be transient and would not be influenced by removal of extracellular Ca^{2+} unless caffeine is applied repeatedly as reported by Chiarandini et al. (1970).

However, the results of the present study demonstrated that caffeine-induced contracture in preparations from normotensive rats has two components: the first rapid contracture followed by the second sustained contracture. The higher concentrations required to initiate the maximum contracture and longer duration of the contracture, even in the first component, were comparable to those reported for toad (Lamb and Stephenson, 1990) and mouse skeletal muscle (Kurebayashi and Ogawa, 2001). Both components of contracture were initiated by an increase in intracellular Ca^{2+} as demonstrated by the fura-2 method. As the first component was insensitive to extracellular Ca^{2+} as in other skeletal muscle, it was assumed that this component of caffeine-induced contracture was initiated by Ca^{2+} released from sarcoplasmic reticulum. Since caffeine, 100 mM, induced no further increase in tension when it was applied cumulatively, it is indicated that almost all of the stored Ca^{2+} in sarcoplasmic reticulum would be released by 30 mM caffeine, i.e. complete depletion. Abolition of the first component by prevention of refilling of sarcoplasmic reticulum with ryanodine (Oyamada et al., 1993) supported this conclusion. The second component of the contracture of this preparation, on the other hand, was sensitive to extracellular Ca^{2+} and was assumed to be brought about by the influx of extracellular Ca^{2+}. In support of this possibility, the addition of Ca^{2+} to Ca^{2+}-depleted preparations in the presence of caffeine in Ca^{2+}-free solution induced contracture, which was recognized from its time course to be the second phase. The observation that this contracture was not blocked by ryanodine, whereas the first component was completely blocked by ryanodine treatment, also indicates that the first component of caffeine-induced contracture was induced by Ca^{2+} released from sarcoplasmic reticulum and the second component was induced by Ca^{2+} that entered from extracellular space. As the second component of contracture was not blocked by verapamil, the involvement of voltage-dependent L-type Ca^{2+} channels could be excluded. In addition, changes in Ca^{2+} sensitivity of contractile elements would be brought about by caffeine especially during the second phase of the contracture as suggested from the relationship between intracellular Ca^{2+} level and contraction height shown in Fig. 5C.

The finding that the resting membrane potential was not different between preparations from WKY and SHRSP was in agreement with the results obtained with some vascular

smooth muscles but disagreed with the result for other vascular smooth muscles (Sunano et al., 2000). The slight membrane depolarization of the preparation from WKY may be explained by an activation of Na^+–Ca^{2+} exchanger and rise of the external K^+ in a restricted space as reported by Lotsias and Venosa (2001) for frog muscle. Then, the lack of depolarization by caffeine in the preparation from SHRSP may be explained by a reduced effect of caffeine on these mechanisms or a lesser contribution of the mechanism to membrane potential. Although the depolarization was not sufficient to induce contraction as proved by increasing the external K^+ concentration, it is still possible that a change in the effect of caffeine on membrane potential is involved in the difference of the second component of contracture.

Wayman et al. (1996) reported that caffeine induced an influx of Ca^{2+} through channels activated by the depletion of Ca^{2+} from sarcoplasmic reticulum in mouse anococcygeus muscle. They reported that the second sustained inward current elicited by caffeine was similar to those elicited by cyclopiazonic acid and was blocked by SK&F 96365, which has been reported to inhibit Ca^{2+} depletion-activated Ca^{2+} currents in some cell types (Leung and Kwan, 1999), but not by nifedipine, an L-type Ca^{2+} channel blocker. The biphasic effect of caffeine on the oesophageal striated muscle resembled the effects of cyclopiazonic acid on mouse anococcygeus muscle (Wayman et al., 1996) and also the effect of caffeine-activated Ca^{2+} influx in growth arrested smooth muscle cells (Ufret-Vincenty et al., 1995). It has also been reported that caffeine initiated an inward Ca^{2+} current by depleting intracellular Ca^{2+} stores in vascular smooth muscle and heart muscle (Baro et al., 1993). However, SK&F 96365 did not block the second component of caffeine-induced contracture in oesophageal striated muscle. LOE 908, which has also been reported to block Ca^{2+} depletion-activated Ca^{2+} currents in human endothelial cells (Encabo et al., 1996), also did not block the second component. The involvement of nonselective cation channels 1 and 2 reported for A7r5 cells, a cultured aorta smooth muscle (Iwamuro et al., 1999) can be less readily confirmed, as LOE 908 and SK&F 96365 showed no effect on the second phase of contracture in these preparations. Recently, Kurebayashi and Ogawa (2001) reported that sarcoplasmic reticulum Ca^{2+} depletion-activated Ca^{2+} influx is seen in skeletal muscle and is inhibited by a high concentration (5 mM) of Ni^{2+} but not by nifedipine, an L-type Ca^{2+} channel blocker. The depression of the second component of caffeine-induced contracture by a high concentration of Ni^{2+} but not by verapamil, observed in the present experiment, may be explained by the inhibition of the Ca^{2+} depletion-activated Ca^{2+} influx as reported for mast cells (Hoth and Penner, 1993), HL-60 cells (Demaurex et al., 1992) and T lymphocytes (Zweifach and Lewis, 1993). Thus, it is possible that the second component of caffeine-induced contracture of the oesophageal striated muscle is initiated by a Ca^{2+} influx through channels activated by the depletion of Ca^{2+} from sarcoplasmic reticulum by caffeine, which is resistant to both SK&F 96365 and LOE 908.

It is of interest that the preparations from SHRSP exhibited a markedly small or no second component of contracture in response to caffeine at the same concentration (30 mM). As the second component of the elevation of intracellular Ca^{2+} level by caffeine was much smaller in the preparations from SHRSP than in those from WKY, the caffeine-induced Ca^{2+} influx may not be sufficient to initiate the tension development required to form the second component in SHRSP preparations. The first component of caffeine-induced contracture in the preparations was initiated by the Ca^{2+} released from sarcoplasmic reticulum, as it showed characteristics similar to those of the first component in the preparations from WKY; it was relatively insensitive to extracellular Ca^{2+} but blocked by ryanodine. The weaker second component in these preparations may be caused by changes in the Ca^{2+} entry mechanism activated directly by caffeine (Guerrero et al., 1994) or by depletion of the Ca^{2+} from sarcoplasmic reticulum by caffeine (Ufret-Vincenty et al., 1995). The difference in the second component of caffeine-induced contracture between the oesophageal striated muscles from WKY and SHRSP resembled that of cyclopiazonic acid-induced contracture of the same preparations, which we reported previously (Sekiguchi et al., 1999), although cyclopiazonic acid did not induce the first rapid contracture, and changes in novel nonselective cation channels, which led to influx of Ca^{2+}, were observed in the preparations from SHRSP. The inability to initiate contracture (the second component) by reapplication of Ca^{2+} to Ca^{2+}-free Tyrode's solution in the presence of caffeine in the preparations from SHRSP also indicated that the mechanism of Ca^{2+} entry activated by caffeine was altered in these preparations. In addition, the difference in caffeine-induced change in Ca^{2+} sensitivity of contractile elements during the second phase of contraction (Fig. 5) may be involved at least in part in the difference in the initiation of the second component of contracture.

It is possible that the muscle type is altered in SHRSP esophagus as reported for the soleus muscle of SHR (Bortolotto et al., 2001; Carlsen and Gray, 1987; Bachir-Lamrini et al., 1990; Gray et al., 1994). However, the threshold caffeine concentration required for the initiation of contracture was higher in the preparations from WKY, differently from that reported in the soleus muscle (Bortolotto et al., 2001). In addition, the large second component such as that observed in the oesophageal striated muscle from WKY with 30 mM caffeine was not observed at any concentration of caffeine up to 100 mM in the preparations from SHRSP, indicating that the difference in response between the oesophageal striated muscles from WKY and SHRSP was not brought about by the difference in caffeine sensitivity due to the difference in the type of muscle. However, it is still possible that the change in the sensitivity of sarcoplasmic reticulum to caffeine is at least partly involved, since the first component was smaller at 30 mM in the preparations from SHRSP, although

it became similar when the caffeine concentration was increased up to 100 mM.

In conclusion, this is the first report on the effects of caffeine on oesophageal striated muscle as far as we found in MEDLINE. We reported here that caffeine, 30 mM, induced a contracture with two components of contraction in the preparations from normotensive WKY. The first component was initiated by Ca^{2+} released from sarcoplasmic reticulum, and the second component was due to Ca^{2+} entry through novel channels activated by Ca^{2+} depletion of sarcoplasmic reticulum or directly by caffeine. We also observed that the second component of caffeine-induced contracture of the oesophageal striated muscle was markedly smaller in the preparations from SHRSP than in those from WKY probably due to the change in Ca^{2+} depletion-activated Ca^{2+} influx. These results also indicate that Ca^{2+} handling properties of cell membrane and sarcoplasmic reticulum are generally altered in muscles of SHRSP.

References

Altman, J., Da Ponte, F., Worcel, M., 1976. Altered reactivity of stomach fundus smooth muscle in Okamoto spontaneous hypertension. Br. J. Pharmacol. 58, 432P–433P.

Atrakchi, A., Gray, S.D., Carlsen, R.C., 1994. Development of soleus muscles in SHR: relationship of muscle deficits to rise in blood pressure. Am. J. Physiol. 267, C824–C835.

Bachir-Lamrini, B.L., Sempore, B., Mayet, M.H., Favier, R.J., 1990. Evidence of a slow-to-fast fiber type transition in skeletal muscle from spontaneously hypertensive rats. Am. J. Physiol. 258, R352–R357.

Baro, I., O'Neill, S.C., Eisner, D.A., 1993. Changes of intracellular [Ca^{2+}] during refilling of sarcoplasmic reticulum in rat ventricular and vascular smooth muscle. J. Physiol. 465, 21–41.

Bortolotto, S.K., Stephenson, D.G., Stephenson, G.M.M., 2001. Caffeine thresholds for contraction in electropholetically typed, mechanically skinned muscle fibres from SHR and WKY rats. Pflügers Arch. 441, 672–700.

Carlsen, R.C., Gray, S.D., 1987. Decline of isometric force and fatigue resistance in skeletal muscles from spontaneously hypertensive rats. Exp. Neurol. 95, 249–264.

Carlsen, R.C., Gray, S.D., Pickar, J.G., 1996a. Na^+, K^+-pump activity and skeletal muscle contractile deficits in the spontaneously hypertensive rat. Acta Physiol. Scand. 156, 237–245.

Carlsen, R.C., Kerlin, D., Gray, S.D., 1996b. Regeneration and revascularization of a nerve-intact skeletal muscle graft in the spontaneously hypertensive rat. Am. J. Physiol. 270, R153–R161.

Chiarandini, D.J., Reuben, J.P., Brandt, P.W., Grundfest, H., 1970. Effects of caffeine on crayfish muscle fibers. J. Gen. Physiol. 55, 640–664.

Demaurex, N., Lew, D.P., Krause, K.-H., 1992. Cyclopiazonic acid depletes intracellular Ca^{2+} stores and activates an influx pathway for divalent cation in HL-60 cell. J. Biol. Chem. 267, 2318–2324.

Duke, A.M., Steele, D.S., 1998. Effects of caffeine and adenine nucleotides on Ca^{2+} release by the sarcoplasmic reticulum in saponin-permeabilized frog skeletal muscle fibres. J. Physiol. 513, 43–53.

Encabo, A., Romanin, C., Birke, F.W., Kukovetz, W.R., Groschner, K., 1996. Inhibition of a store-operated Ca^{2+} entry pathway in human endothelial cells by the isoquinoline derivative LOE 908. Br. J. Pharmacol. 119, 702–706.

Endo, M., 1977. Calcium release from the sarcoplasmic reticulum. Physiol. Rev. 57, 71–108.

Endo, M., Tanaka, M., Ogawa, Y., 1970. Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibers. Nature 228, 34–36.

Fleischer, S., Inui, M., 1989. Biochemistry and biophysics of excitation–contraction coupling. Annu. Rev. Biophys. Biophys. Chem. 18, 333–364.

Gray, S.D., Carlsen, R.C., Atherley, R., 1994. Effect of chronic blood pressure reduction on soleus muscle contractile properties in spontaneously hypertensive rats. Am. J. Physiol. 267, R740–R746.

Gruber, H., 1968. Über Struktur und Innervation der quergestreiften Muskulature des Oesophagus der Ratte. Z. Zellforsch. 91, 236–247.

Guerrero, A., Fay, F.C., Singer, J.J., 1994. Caffeine activates a Ca^{2+}-permeable nonselective cation channel in smooth muscle cells. J. Gen. Physiol. 104, 375–394.

Hoth, M., Penner, R., 1993. Calcium release-activated calcium current in rat mast cells. J. Physiol. 465, 359–386.

Iwamuro, Y., Miwa, S., Zhang, X.-F., Minowa, T., Enoki, T., Okamoto, Y., Hasagawa, H., Furutani, H., Okazawa, M., Ishikawa, M., Hashimoto, N., Masaki, T., 1999. Activation of three types of voltage-independent Ca^{2+} channel in A7r5 cells by endothelin-1 as revealed by a novel Ca^{2+} channel blocker LOE 908. Br. J. Pharmacol. 126, 1107–1114.

Kanagy, N.L., Ansari, M.N., Ghosh, S., Webb, R.C., 1994. Recycling and buffering of intracellular calcium in vascular smooth muscle from genetically hypertensive rats. J. Hypertens. 12, 1365–1372.

Kawata, K., Sekiguchi, F., Shimamura, K., Sunano, S., 2000. Effects of caffeine on twitch contraction and basal tension in the esophageal striated muscle from hypertensive and normotensive rats. Jpn. J. Pharmacol. 82 (Suppl. 1), 211P.

Kurebayashi, N., Ogawa, Y., 2001. Depletion of Ca^{2+} in the sarcoplasmic reticulum stimulates Ca^{2+} entry into mouse skeletal muscle fibres. J. Physiol. 533, 185–199.

Kwan, C.Y., Grover, A.K., Sakai, Y., 1982. Abnormal biochemistry of subcellular membranes isolated from nonvascular smooth muscles of spontaneously hypertensive rats. Blood Vessels 19, 273–283.

Lamb, G.D., Stephenson, D.G., 1990. Control of calcium release and the effect of ryanodine in skinned muscle fibres of the toad. J. Physiol. 42, 519–542.

Leung, Y.M., Kwan, C.Y., 1999. Current perspectives in the pharmacological studies of store-operated Ca^{2+} entry blockers. Jpn. J. Pharmacol. 81, 253–258.

Lotsias, B.A., Venosa, R.A., 2001. Caffeine-induced depolarization in amphibian skeletal muscle fibres: role of Na^+/Ca^{2+} exchanger and K^+ release. Acta Physiol. Scand. 171, 459–466.

Moriyama, K., Osugi, S., Shimamura, K., Sunano, S., 1989. Caffeine-induced contraction in arteries from stroke-prone spontaneously hypertensive rats. Blood Vessels 26, 280–289.

Oyamada, H., Iino, M., Endo, M., 1993. Effects of ryanodine on the properties of Ca^{2+} release from the sarcoplasmic reticulum in skinned skeletal muscle fibres of the frog. J. Physiol. 470, 335–348.

Pickar, J.G., Carlsen, R.C., Atrakchi, A., Gray, S.D., 1994. Increased Na^+–K^+ pump number and decreased pump activity in soleus muscles in SHR. Am. J. Physiol. 267, C836–C844.

Ríos, E., Pizarro, G., 1991. Voltage sensor of excitation–contraction coupling in skeletal muscle. Physiol. Rev. 71, 849–908.

Rousseau, F., LaDine, J., Liu, Q.-Y., Meissner, G., 1988. Activation of the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum by caffeine and related compounds. Arch. Biochem. Biophys. 15, 75–86.

Salviati, G., Volpe, P., 1988. Ca^{2+} release from sarcoplasmic reticulum of skeletal fast- and slow-twitch muscle fibers. Am. J. Physiol. 254, C459–C465.

Sandow, A., 1965. Excitation–contraction coupling in skeletal muscle. Pharmacol. Rev. 17, 265–320.

Sekiguchi, F., Shimamura, K., Kawata, K., Nakazawa, Y., Saitoh, R., Yanagitani, Y., Sunano, S., 1999. Effects of cyclopiazonic acid on contraction and intracellular Ca^{2+} in oesophageal striated muscle of normotensive and spontaneously hypertensive rats. Br. J. Pharmacol. 128, 961–968.

Sekiguchi, F., Kawata, K., Shimamura, K., Sunano, S., 2003. Reduced

effect of caffeine on twitch contraction of oesophageal striated muscle from stroke-prone spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. (in press).

Su, J.Y., 1988. Mechanisms of ryanodine-induced depression of caffeine-induced tension transients in skinned striated rabbit muscle fibers. Pflügers Arch. 411, 371–377.

Sugiyama, T., Yoshizumi, M., Takaku, F., Yazaki, Y., 1990. Abnormal calcium handling in vascular smooth muscle cells of spontaneously hypertensive rats. J. Hypertens. 8, 369–375.

Sunano, S., Sekiguchi, F., Shimamura, K., 2000. Arterial smooth muscle tone and its modification by endothelium in spontaneously hypertensive rats. Biomed. Res. 11, 137–147.

Syme, P.D., Arnolda, L., Green, Y., Aronson, J.K., Grahame-Smith, D.G., Radda, G.K., 1990. Evidence for increased in vivo $Na^{+}-H^{+}$ antiporter activity and an altered skeletal muscle contractile response in the spontaneously hypertensive rat. J. Hypertens. 8, 1027–1036.

Ufret-Vincenty, C.A., Short, A.D., Alfonso, A., Gill, D.L., 1995. A novel Ca^{2+} entry mechanism is turned on during growth arrest induced by Ca^{2+} pool depletion. J. Biol. Chem. 270, 26790–26793.

Wayman, C.P., McFadzean, I., Gibson, A., Tucker, J.F., 1996. Two distinct membrane currents activated by cyclopiazonic acid-induced calcium store depletion in single smooth muscle cells of the mouse anococcygeus. Br. J. Pharmacol. 117, 566–572.

Zweifach, A., Lewis, R.S., 1993. Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} store. Proc. Natl. Acad. Sci. U. S. A. 90, 6295–6299.