Original Article

Changes in Intracellular Ca^{2+} Induced with Adrenaline in Swine Lingual Artery

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ABSTRACT

Background and Objectives: Dental doctors routinely infiltrate adrenaline combined with lidocaine into the oral mucosa. However, they do not well know the physiological characteristics and mechanisms of contraction induced by adrenaline in the oral maxillofacial artery. We investigated the changes in the intracellular Ca^{2+} concentration by an adrenaline-induced contraction in the swine lingual artery. **Materials and Methods:** We prepared artery rings with denuded endothelium and simultaneously measured tension and intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). The effects of adrenaline in the presence or absence of intracellular Ca^{2+} on artery rings and verapamil on Ca^{2+} influx activated by adrenaline were assessed. **Results:** In the presence of intracellular Ca^{2+}, the application of adrenaline caused a rapid increase in tension and $[Ca^{2+}$ i, which then decreased slowly. In the absence of extracellular Ca^{2+}, adrenaline caused a transient increase in $[Ca^{2+}]i$ and tension. The application of adrenaline in the presence of extracellular Ca^{2+} after depletion of the intracellular Ca^{2+} store caused a slow increase in $[Ca^{2+}]i$ and tension, while co-treatment with verapamil inhibited the increases in $[Ca^{2+}]$intracellular and tension was induced by adrenaline. The tension relationship obtained with adrenaline was located on the left of the $[Ca^{2+}]i$-tension relation curve obtained with KCl. **Conclusions:** The induction of contraction may involve three mechanisms: (1) Release of Ca^{2+} from the intracellular store, (2) influx of extracellular Ca^{2+} through voltage-gated Ca^{2+} channels, and (3) the Ca^{2+} sensitivity of the contractile apparatus.

Key words: Adrenaline, Ca^{2+} sensitivity of the contractile apparatus, influx of extracellular Ca^{2+} channel, intracellular Ca^{2+} store, verapamil

INTRODUCTION

Vasoconstrictor agents administered concomitantly with local anesthetic are used to decrease blood loss, maintain clear visualization, slow the rate of absorption, reduce toxicity, and prolong duration.[1-3] Adrenaline combined with lidocaine is widely used for dental treatment and the oral and maxillofacial surgery. Although dental doctors commonly infiltrate adrenaline combined with lidocaine into the oral mucosa during extraction, minor surgery, or periodontal surgery with bleeding, they do not well know the physiological characteristics and mechanisms of contraction induced by adrenaline in the oral maxillofacial artery.

Among the craniofacial arteries, the lingual artery is relatively unexplored despite being a prominent artery that supplies the tongue, a pivotal organ for oral functions including tasting, mastication, swallowing, and speech. Characterization of the contraction of the lingual artery is required to assess common features to and distinguished from those of other vascular supply, which may have implications for the clinical treatment of any pathological circulation in the tongue.[4,5] In the present study, since the contraction of vascular smooth muscle is regulated by

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changes in cytosolic (intracellular) Ca^{2+} levels ($[Ca^{2+}]i$) and Ca^{2+} sensitivity of contractile elements,[6] we investigated the changes in intracellular Ca^{2+} concentration by adrenaline-induced contraction in the swine lingual artery with denuded endothelium by simultaneously measuring tension and $[Ca^{2+}]i$ using front-surface fluorometry and the Ca^{2+} indicator dye fura-2/AM. First, we assessed the effect of adrenaline and verapamil on contractions of endothelium-denuded rings. Next, we observed the effect of adrenaline and verapamil on the Ca^{2+} influx in endothelium-denuded rings in a Ca^{2+}-free solution.

MATERIALS AND METHODS

This research was ethically approved by the Institutional Review of Committee on the Ethics of Animal Experiments of Iwate Medical University. All experiments were carried in agreement with the Institutional Animals Care and under given Committee guidelines (approval number #26-010).

Reagents and Solutions

L-adrenaline bitartrate was obtained from regional city Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All other chemicals required for the research were obtained from industry name Wako Pure Chemicals (Osaka, Japan). In all investigations, air-equilibrated Hanks' balanced salt solution (HBSS) was used to maintain the artery under resting conditions. HBSS (used to maintain the osmotic pressure and pH in cells) consisted of 137 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 1.26 mM $CaCl_2$, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, and 5.55 mM glucose (pH 7.34). All other SSs used as perfusate were made by modifying HBSS. (http://bbbulletin.org/index.php/BBB/article/download/167/146) SS (100 mM) was made by substituting KCl for the equivalent concentration of NaCl in HBSS. Ca^{2+}-free SS was made by adding GEDTA (to 1 mM) and omitting CaCl2 from HBSS. Each SS containing adrenaline, caffeine, ryanodine, and verapamil alone or in combination was made by adding the respective components into base SS or Ca^{2+}-free SS immediately before use.

Artery Rings Preparation and Contraction Measurement

In swine tongues, segments of lingual artery were prepared, and isometric tension were measured as described in detail previously.[4,5] Fresh swine tongues were obtained at a local abattoir. A segment of the lingual artery at the proximal region of the tongue was excised. After adventitia was removed, the artery segments (~2 mm in diameter) were cut into 2-mm-long rings, and the lumen surface was rubbed gently against a thin arm of stainless steel tweezers to remove the endothelium. An artery ring was held with two tungsten needles in the perfusion chamber (perfusate holding a volume of 2.0 ml). One needle was fastened to a displacement transducer (Type UL-2GR; Minebea Co., Fujisawa, Japan) and the other to a micromanipulator. The solution was bubbled with a mixture of 95% O_2 and 5% CO_2 and held at 37°C and flow rate of 1.6 ml/min with a peristaltic pump (SMP-23; Tokyo Rikakikai Co., Tokyo, Japan). As the strength of contraction did not change when the resting tone was 3-7 mN, the artery rings were extended to give a resting tone of approximately 5 mN, And then artery rings were perfused with 100 mM KCl for 5 min as a control. After a 10-20 min perfusion with HBSS to resting position, artery rings were perfused with HBSS or Ca^{2+}-free SS plus a stimulant for 5 or 15 min. As the contraction was considered to show the essential features of contraction under the stimulation conditions, the effects of ryanodine, verapamil, and extracellular Ca^{2+} were examined in the perfusion subsequent to the contraction. The contraction was detected as an increase in isometric tension with the displacement transducer, and detected signals were amplified with a carrier amplifier (CSD-815; Digital indicator, Minebea Co., Fujisawa, Japan) and recorded with a Powerlab 16/30T data acquisition system (Australia AD Instruments). The strengths of any contractions in an experiment were normalized to the strength of the 100 mM KCl contraction and expressed as percentages.

Fura-2 Loading

Artery rings were loaded with the Ca^{2+} indicator dye, fura-2, by incubating in HBSS containing 10 μM fura-2/AM (an acetoxymethyl ester form of fura-2) for 3 h at 37°C in the dark. After loading with fura-2, the rings were washed with HBSS to remove the dye in the extracellular space and were then kept in HBSS for at least 1 h before starting the measurements.

Measurement of Fura-2 Fluorescence

Changes in fluorescence intensity of the fura-2-Ca^{2+} complex were monitored using a front-surface fura-2 fluorometer (Aquacosmos; Hamamatsu Photonics K.K., Tokyo, Japan). Two wavelengths of excitation light (340 and 380 nm) were obtained spectroscopically from a Xenon light source. Rings were illuminated by guiding the two alternating wavelengths of excitation light through quartz optical fibers. Surface fluorescence of the ring was collected by glass optical fibers and introduced through a 500 nm emission light, which was induced by alternating two wavelengths of excitation light (340 nm and 380 nm). The ratio of the fluorescence intensities (fluorescence ratio) at 340 nm excitation (F340) to that at 380 nm excitation (F380) was monitored to estimate changes in $[Ca^{2+}]i$. Data were expressed as a percentage, while the values at rest in HBSS (5.84 mM

K+) and 100 mM KCl were designated as 0% and 100%, respectively.

Simultaneous Measurement of Tension and Ratio

Effects of adrenaline on rings with denuded endothelium

After a 5 min 100 mM KCl perfusion had performed to confirm that the artery ring retained intact contractility throughout the experiment, a 0.5, 1.0, 5.0, 10, or 100 μM adrenaline was perfused for 5 min each and expressed as a percentage. The strengths of any contractions and changes in $[Ca^{2+}]$i in an experiment were normalized to the strength of a 100 mM KCl contraction and the fluorescence ratio, and expressed as a percentage.

Effects of verapamil on the adrenaline-induced contraction

After a 5-min 100 mM KCl perfusion was performed to confirm that the artery ring retained intact contractility throughout the experiment, 5 μM adrenaline plus 0.01, 0.1, 1.0, 10, or 100 μM verapamil was perfused for 5 min each and expressed as a percentage. The strengths of any contractions and changes in $[Ca^{2+}]$i in an experiment were normalized to the strength of a 5 μM adrenaline contraction and the fluorescence ratio, and expressed as a percentage.

Effects of adrenaline in the presence or absence of extracellular Ca^{2+} on rings with denuded endothelium

After a 5-min 100 mM KCl perfusion was performed to confirm that the artery ring retained intact contractility throughout the experiment, 5 μM adrenaline in Ca^{2+}-free SS or SS was perfused for 5 min each and expressed as a percentage. The strengths of any contractions and changes in $[Ca^{2+}]$i in an experiment were normalized to the strength of a 100 mM KCl contraction and the fluorescence ratio, and expressed as a percentage.

Effects of adrenaline in the absence of the extracellular Ca^{2+} after depletion of the intracellular Ca^{2+} store on rings with denuded endothelium

After a 5-min 100 mM KCl perfusion had been performed to confirm that the artery ring retained intact contractility throughout the experiment, experiments were performed on the rings in Ca^{2+}-free SS containing 1 mM EDTA for 5 min. Caffeine (25 mM) was applied 3 times in the same Ca^{2+}-free SS to remove Ca^{2+} in the intracellular Ca^{2+} store. The 25 mM "first caffeine" was applied for 5 min in Ca^{2+}-free SS, followed by washout with Ca^{2+}-free SS. After washout with Ca^{2+}-free SS, 30 μM ryanodine was applied with Ca^{2+}-free SS for 5 min, followed by "second caffeine" applied for 5 min. After washout with Ca2+-free SS for 5 min, the "third caffeine" was applied for 5 min and then washout for 5 min using Ca^{2+}-free SS. Subsequently, adrenaline in Ca^{2+}-free SS was applied for 15 min and expressed as a percentage. Strengths of any contractions and changes in $[Ca^{2+}]$i in an experiment were normalized to the strength of a 100 mM KCl contraction and fluorescence ratio and expressed as a percentage.

Effects of adrenaline with or without verapamil in the presence of extracellular Ca^{2+} after depletion of the intracellular Ca^{2+} store on rings with denuded endothelium

After a 5-min 100 mM KCl perfusion had been performed to confirm that the artery ring retained intact contractility throughout the experiment, ring experiments were performed in Ca^{2+}-free SS containing 1 mM EDTA for 5 min. Caffeine (25 mM) was applied 3 times in the same Ca^{2+}-free SS to remove Ca^{2+} in the intracellular Ca^{2+} store. A 25-mM "first caffeine" was applied for 5 min in Ca^{2+}-free SS, followed by washout with Ca^{2+}-free SS. Ryanodine (30 μM) was then applied with Ca^{2+}-free SS for 5 min, followed by "second caffeine" applied for 5 min. After washout with Ca^{2+}-free SS for 5 min, the "third caffeine" was implemented for 5 min, followed by washout for 5 min using Ca^{2+}-free SS. Subsequently, adrenaline in Ca^{2+}-free SS or HBSS (Ca^{2+} in) was applied for 15 min and expressed as a percentage. The strengths of any contractions and changes in $[Ca^{2+}]$i in an experiment were normalized to the strength of a 100 mM KCl contraction and the fluorescence ratio, and expressed as a percentage.

Statistical Analysis

Values are presented as a mean ± standard error of mean. The means difference in the two groups were evaluated by Student's *t*-test. Differences between two multiple groups, whose homogeneity of variance was assessed by the Levene test were evaluated by using Dunnet's *t*-test. Differences were considered significant at $P < 0.05$.

RESULTS

Effects of Adrenaline on Rings with Denuded Endothelium

Adrenaline (0.5 μM-1 mM) induced a concentration-dependent contraction and an increase in $[Ca^{2+}]$i of lingual swine arterial rings with denuded endothelium [Figure 1].

Effects of Verapamil on the Contraction Induced by Adrenaline

Verapamil produced a dose-dependent attenuation of the contraction and an increase in $[Ca^{2+}]$i induced by adrenaline [Figure 2].

Effects of Adrenaline in the Presence or Absence of Extracellular Ca^{2+} on Rings with Denuded Endothelium

Representative time courses of $[Ca^{2+}]i$ and tension development induced by application of 100 mM KCl and 5 μM adrenaline in Ca^{2+}-free SS or HBSS are shown in Figure 3a and b. Application of 5 μM adrenaline for 5 min in the presence of extracellular Ca^{2+} caused a rapid increase in $[Ca^{2+}]i$ (peak increase 59 ± 1%; n = 8) and tension (peak increase 77 ± 2%; n = 8) [Table 1], which then decreased slowly thereafter. Application of 5 μM adrenaline for 5 min in the absence of extracellular Ca^{2+} caused a rapid and transient increase in $[Ca^{2+}]i$ (peak increase 52 ± 2%; n = 8) and tension (71 ± 3%, n = 8) [Table 1].

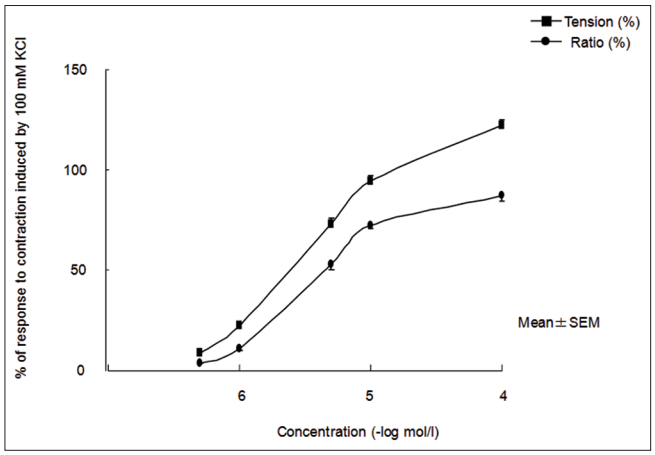

Figure 1: Dose-response effects of adrenaline on the swine lingual artery. Adrenaline induced a concentration-dependent contraction and an increase in intracellular Ca^{2+} concentration of swine lingual arterial rings with denuded endothelium (n=8). Data are mean ± standard error of mean

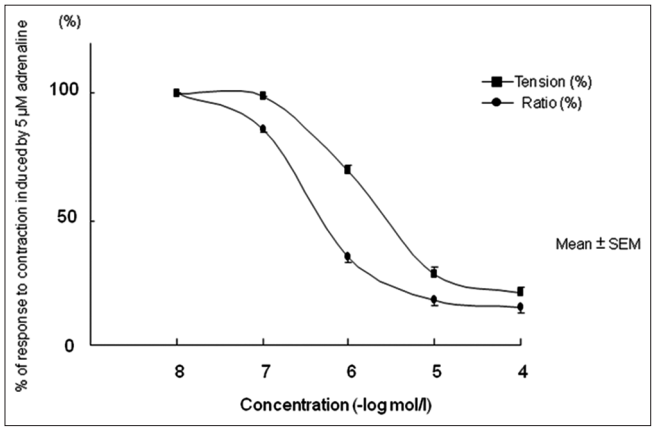

Figure 2: Dose-response effects of verapamil on contraction induced by 5 μM adrenaline. Verapamil produced a dose-dependent attenuation of the contraction and an increase in intracellular Ca^{2+} concentration induced by adrenaline (n = 8). Data are mean ± standard error of mean

Effects of Adrenaline in the Absence of Extracellular Ca^{2+} after Depletion of the Intracellular Ca^{2+} Store on Rings with Denuded Endothelium

Representative time courses of $[Ca^{2+}]i$ and tension development induced by application of 100 mM KCl and 5 μM adrenaline in Ca^{2+}-free SS and after depletion of the intracellular Ca^{2+} store are shown in Figure 4. Application of 5 μM adrenaline for 5 min in the absence of extracellular Ca^{2+} and intracellular Ca^{2+} store produced no response [Table 1].

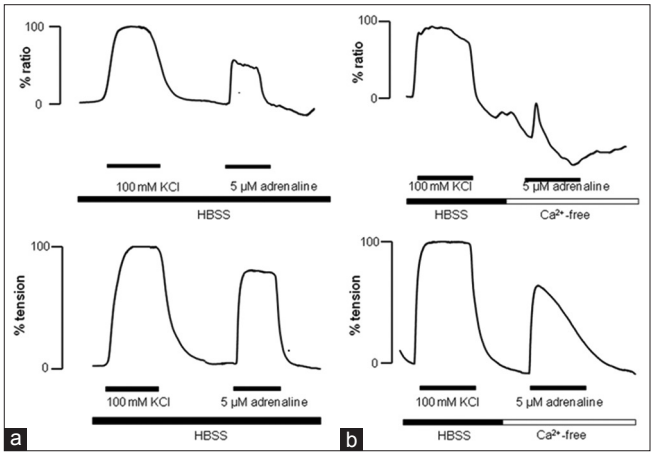

Figure 3: Effects of adrenaline on the lingual artery in the presence or absence of extracellular Ca^{2+}, (a) Presence of intracellular Ca^{2+} and extracellular Ca^{2+}, (b) Presence of intracellular Ca^{2+} and absence of extracellular Ca^{2+}. The application of 5 μM adrenaline for 5 min in the presence of extracellular Ca^{2+} caused a rapidly increase in the intracellular Ca^{2+} concentration (**a1**) and tension (**a2**), which then slowly reduced (n = 8). Application of 5 μM adrenaline for 5 min in the absence of extracellular Ca^{2+} caused a transient increase in intracellular Ca^{2+} concentration (**b1**) and tension (**b**2) (n = 8)

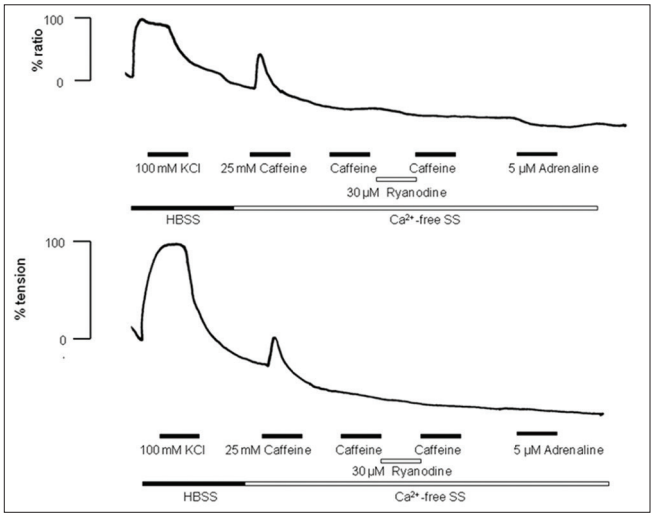

Figure 4: Effects of adrenaline on the lingual artery in the absence of intracellular Ca^{2+} and extracellular Ca^{2+}. Application of 5 μM adrenaline for 5 min in the absence of extracellular Ca^{2+} and intracellular Ca^{2+} store produced no response (n = 8)

Table 1: Effects of adrenaline on the lingual artery in the presence or absence of intra- or extracellular Ca^{2+}

Parameters	Intracellular Ca^{2+} (+); extracellular Ca^{2+} (+)	Intracellular Ca^{2+} (+); extracellular Ca^{2+} (–)	Intracellular Ca^{2+} (–); extracellular Ca^{2+} (–)
Ratio (%)	59±1	52±2	No response
Tension (%)	77±2	71±3	No response

Values are mean±SEM. n=8 per group. SEM: Standard error of mean, +: Presence, –: Absence

Effects of adrenaline with and without verapamil in the presence of extracellular Ca^{2+} after depletion of the intracellular Ca^{2+} store on rings with denuded endothelium. Representative time courses of $[Ca^{2+}]i$ and tension development induced by application of 100 mM KCl and 5 μM adrenaline in HBSS and after depletion of the intracellular Ca^{2+} store are shown in Figure 5. Application of 5 μM adrenaline for 15 min in the present of extracellular Ca^{2+} caused a slow increase in $[Ca^{2+}]i$ (peak increase 48 ± 3%; n = 8) and tension (peak increase 63 ± 3%; n = 8). Treatment with 10 μM verapamil 5 min before and during the application of adrenaline significantly inhibited the increase in $[Ca^{2+}]i$ (peak increase 30 ± 3%; n = 8) and tension (peak increase 31 ± 2%; n = 8) induced by application of adrenaline [Figure 5b and Table 2].

Relationship between changes in $[Ca^{2+}]i$ and tension induced by adrenaline and KCl The $[Ca^{2+}]i$–tension relationship obtained with adrenaline was located on the left of the $[Ca^{2+}]i$–tension relation curve obtained with KCl, indicating the Ca^{2+} sensitizing effect of adrenaline.

Table 2: Effects of verapamil on contraction induced by adrenaline in the presence of extracellular Ca^{2+} after depletion of intracellular Ca^{2+} store

Parameters	Adrenaline	Adrenaline+Verapamil
Ratio (%)	48±3	30±3
Tension (%)	63±3	31±2*

Values are mean±SEM. *P<0.05 versus adrenaline (control). n=8 per group, SEM: Standard error of mean

DISCUSSION

The main findings of the present study were that the contraction induced by adrenaline in the swine lingual artery is dependent on the increase in $[Ca^{2+}]i$., and that verapamil attenuates the increase in $[Ca^{2+}]i$. Moreover, tension induced by adrenaline. The contraction induced by adrenaline in the swine lingual artery was dependent on the increase in $[Ca^{2+}]$ i. Hence, when examining vascular contraction induced by agonists, it is essential to consider factors such as the vascular bed, stimulant, and contractile type classified as phasic (or fast) or tonic (or slow).[6,7] A contraction consists of two components: The phasic (or fast) contraction appearing as a large increase at the initial part of the contraction, and the tonic (or slow) contraction appearing as a sustained level of force at the delayed phase of contraction.[4] Contraction-induced by an agonist depends on Ca^{2+} release from sarcoplasmic stores.[6] It is generally assumed that the phasic contraction depends on Ca^{2+} release from sarcoplasmic stores,[8] while the tonic contraction depends largely on Ca^{2+} influx through a receptor-activated Ca^{2+} channel or receptor-regulated non-selective cation channel.[9] In the present study, stimulation with adrenaline in HBSS caused a rapid contraction of artery rings to a maximum strength, followed by a slow relaxation (in general, phasic contraction occurred followed by tonic contraction) [Figure 3a]. Stimulation with adrenaline in Ca^{2+}-free SS caused aphasic contraction and a transitory increase in $[Ca^{2+}]i$ [Figure 3b]. This transitory increase in $[Ca^{2+}]i$ is thought to depend on the release of Ca^{2+} from the IP3-channel-operated store, a sarcoplasmic store.[4] Caffeine and ryanodine in Ca^{2+}-free SS were applied before adrenaline in HBSS (Ca^{2+} in) to remove Ca^{2+} from the sarcoplasmic reticulum. Ryanodine (RyRs) receptors are a sarcoplasmic/endoplasmic reticulum Ca^{2+} release channel[10] that keeps the Ca^{2+}-induced Ca^{2+} release channel open.[11] Caffeine can release Ca^{2+} from the common Ca^{2+} store, which can be depleted completely by caffeine.[12] Therefore, after sequential application of ryanodine and caffeine, Ca^{2+} in the intracellular Ca^{2+} store is completely removed.

Stimulation with adrenaline in HBSS (Ca^{2+} in) after depletion of the intracellular Ca^{2+} store did not cause aphasic contraction or transient increase in $[Ca^{2+}]$ I [Figure 5a], suggesting that the transient increase in $[Ca^{2+}]i$ in this phasic contraction depends on the release of Ca^{2+} from the sarcoplasmic reticulum. Thus, stimulation with adrenaline in HBSS (Ca^{2+} in) after depletion of the intracellular Ca^{2+} store caused a slow and small tonic contraction and $[Ca^{2+}]i$ [Figure 5a]. This increase in $[Ca^{2+}]i$ depends on the influx of extracellular Ca^{2+} through voltage-gated Ca^{2+} channels (L- and T-type). Agonist stimulation elicits a larger contraction than does KCl stimulation under similar bulk average concentrations of sarcoplasmic Ca^{2+}. In the present study, the $[Ca^{2+}]i$-tension relation obtained with adrenaline was located on the left of the $[Ca^{2+}]i$–tension relation curve obtained with KCl. Thus, stimulation with adrenaline at a given $[Ca^{2+}]i$ causes a greater contraction than does that with KCl, indicating that the Ca^{2+} sensitivity of the contractile apparatus is increased by adrenaline. In support of this result, use of other agonists was reported to increase myofilament Ca^{2+} sensitivity in other smooth muscles, potentially via protein kinase C activation.[13] However, the mechanism of the adrenaline-induced increase in Ca^{2+} sensitivity of the contractile apparatus in the present study remains unclear [Figure 6].

We also found that verapamil attenuated the increase in $[Ca^{2+}]i$. Moreover, tension induced by adrenaline. Calcium

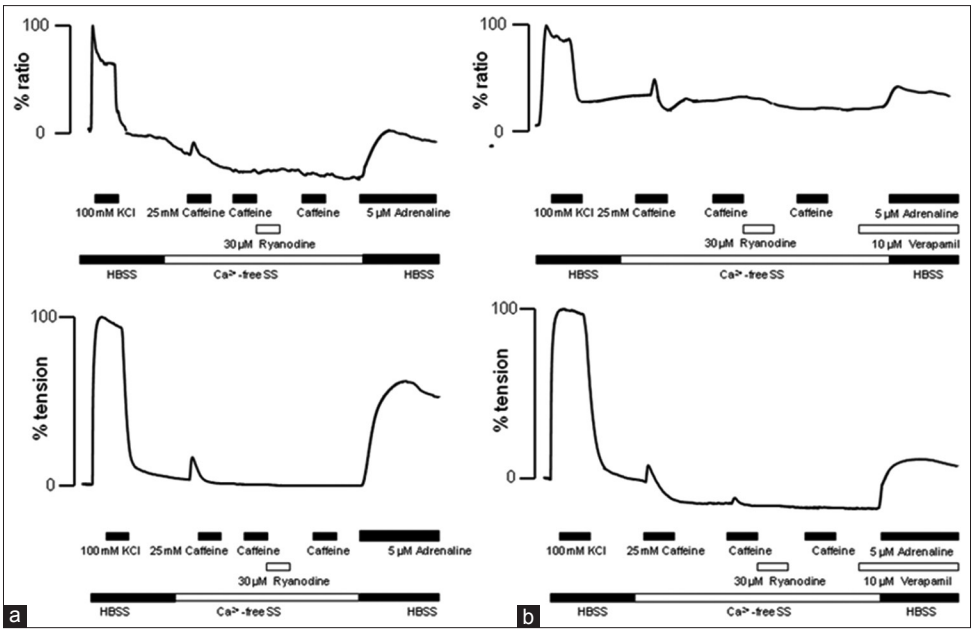

Figure 5: Effects of verapamil on contraction induced by adrenaline in the presence of extracellular Ca^{2+} after depletion of intracellular Ca^{2+}. Application of 5 μM adrenaline for 15 min in the presence of extracellular Ca^{2+} caused a slow increase in the intracellular Ca^{2+} concentration (a1) and tension (a2) ($n = 8$). Treatment with 10 μM verapamil 5 min before and during the application of adrenaline significantly inhibited the increase of intracellular Ca^{2+} (b1) and tension (b2) induced by application of adrenaline ($n = 8$)

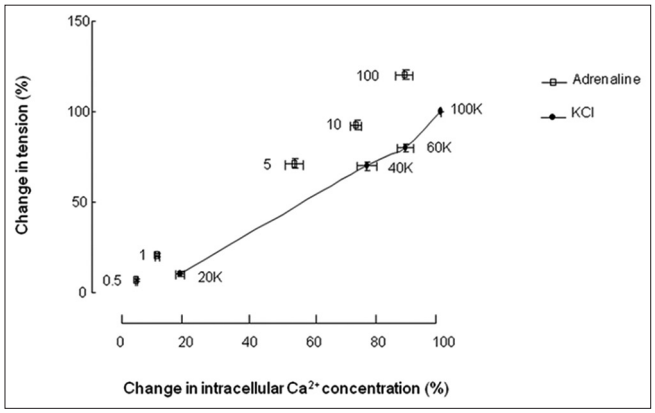

Figure 6: Relationship between changes in tension and intracellular Ca^{2+} concentration induced by adrenaline and KCl. Changes in tension and intracellular Ca^{2+} concentration are normalized as shown in Figure 1. Closed circle: Response to high KCl media ($n = 8$). Open square: Response to adrenaline ($n = 8$). Numbers on the symbols indicate concentrations used: 20 K, 40 K, 60 K, and 100 K represent 20, 40, 60, and 100 mM KCl, respectively; 0.5, 1, 5, 10, and 100 represent 0.5, 1.0, 5.0, 10, and 100 μM adrenaline, respectively. Data are mean ± standard error of mean. The intracellular Ca^{2+} concentration–tension relationship obtained with adrenaline was located on the left of the intracellular Ca^{2+} concentration-tension relation curve obtained with KCl, indicating the Ca^{2+} sensitizing action of adrenaline

influx is the major pathway driving increased $[Ca^{2+}]i$. This mechanism includes voltage-dependent L-type Ca^{2+} channels, non-selective cation channels, the Ca^{2+} release-activated Ca^{2+} influx pathway and reversal of the $Na+/Ca^{2+}$ exchanger [9]. Verapamil is an L-type channel blocker. Verapamil-sensitive Ca^{2+} mobilization, likely via Ca^{2+} influx through L-type Ca^{2+} channels, may provide Ca^{2+} for both the agonist- and the voltage-operated excitation-concentration coupling directly or indirectly[4]. In the present study, verapamil significantly inhibited the adrenaline-induced increase in $[Ca^{2+}]i$ [Figure 5b and Table 2], suggesting that the majority of the adrenaline-induced increase in $[Ca^{2+}]i$. during tonic contraction is dependent on the influx of extracellular Ca^{2+} via the L-type channel.

There are some limitations to this study. First, we cannot be sure that swine lingual artery contractions exhibit the same characteristics as contractions in the human lingual artery. Second, the data in this study are based on a relatively small sample size. Finally, in some of our experiments, only swine lingual artery with denuded endothelium was investigated. Nevertheless, we believe that our data provide accurate and reliable information on the characteristics of contractions in human lingual arteries and that our findings are useful for further investigation of craniofacial arteries. Future studies are required to compare swine lingual arteries with intact endothelium with those with denuded endothelium and further clarify the characteristics of adrenaline-induced contractions.

In this study, the contraction induced by adrenaline in the swine lingual artery is dependent on the increase in $[Ca^{2+}]i.$, while verapamil attenuated the increase in $[Ca^{2+}]i$. And tension induced by adrenaline. Contraction-induced by adrenaline may involve three mechanisms: (1) Release of Ca^{2+} from the intracellular store, (2) influx of extracellular Ca^{2+} through voltage-gated Ca^{2+} channels and (3) the Ca^{2+} sensitivity of the contractile apparatus, which may

be due to protein kinase C activity. It is possible that the L-type extracellular Ca^{2+} channel may be the main channel involved in the contraction induced by adrenaline. These three mechanisms may be common in the contraction induced by agonists. Future studies are required to investigate the mechanisms of dilation induced by local anesthetics including lidocaine or mepivacaine in swine lingual artery.

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