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Differential Contribution of Nerve-Derived Noradrenaline to High K\(^+\)-Induced Contraction Depending on Type of Artery

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High K\(^+\)-induced contraction of arterial smooth muscle is thought to be mediated by membrane depolarization and subsequent activation of voltage-dependent Ca\(^{2+}\) channels (VDCCs). In line with this, this study found that contraction induced by 80 mM K\(^+\) was almost abolished by nifedipine (1 \(\mu\)M), a VDCC inhibitor, in isolated rat aorta, and was markedly suppressed in the iliac artery. However, nifedipine (1 \(\mu\)M) only partially suppressed high K\(^+\)-induced contraction in the tail artery. The contractions remaining in the arteries were further reduced by non-selective cation channel (NSCC) inhibitors, including 2-aminoethoxydiphenyl borate (2-APB) (100 \(\mu\)M), SK&F96365 (10 \(\mu\)M), and 3,4-dihydro-6,7-dimethoxy-\(\alpha\)-phenyl-N,N-bis[2-(2,3,4-trimethoxyphenyl)ethyl]-1-isouquinolineacetamide hydrochloride (LOE908) (10 \(\mu\)M). In particular, sustained tonic contraction was nearly abolished. Prazosin (0.3 \(\mu\)M), an \(\alpha\)-adrenoceptor antagonist, partially inhibited high K\(^+\)-induced contraction in the tail and iliac arteries, but had no effect in the aorta. Consistently, tyramine potently induced contraction in the tail and iliac arteries, but not in the aorta. Furthermore, the inhibition by prazosin and NSCC inhibitors of the high K\(^+\)-induced contraction in the presence of nifedipine was comparable. These results suggest that depending on the type of artery, high K\(^+\)-induced contraction is mediated by Ca\(^{2+}\) influx not only through VDCCs but also through NSCCs, the activation of which is due to the activation of \(\alpha\)-adrenoceptors by the released noradrenalin from sympathetic nerve terminals resulting from high K\(^+\) stimulation.

Key words high K\(^+\); tail artery; non-selective cation channel; voltage dependent calcium channel; noradrenaline

Ca\(^{2+}\) elevation in cytosol causes contraction in smooth muscle. In arterial smooth muscle, the primary pathways of Ca\(^{2+}\) influx from extracellular space are through L-type voltage-dependent Ca\(^{2+}\) channels (VDCCs) and non-selective cation channels (NSCCs).\(^1\) Elevating extracellular K\(^+\) concentration decreases the K\(^+\) gradient between the inside and outside of the cell membrane, which in turn causes cell membrane depolarization. VDCCs are activated by this depolarization, which leads to Ca\(^{2+}\) influx, thereby causing smooth muscle contraction. Therefore, high K\(^+\) stimulation is frequently used as an estimation of VDCC-mediated contraction.\(^2\) It is interesting to note, however, that our preliminary study showed insufficient inhibition of high K\(^+\)-induced contraction by the VDCC inhibitor nifedipine in isolated rat tail artery. We thus further investigated the mechanism underlying high K\(^+\)-induced contraction in different types of arteries, i.e., the aorta, and the iliac and tail arteries. The present data show that, depending on the type of artery, high K\(^+\)-induced contraction is mediated not only through VDCCs but also through NSCCs activated by noradrenaline released from sympathetic nerve terminals.

MATERIALS AND METHODS

Measurement of Contraction Protocols for animal use were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Shizuoka.

Male Wistar rats (8–12 weeks old; SLC, Shizuoka, Japan) were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal (i.p.)), and sacrificed by decapitation. The aorta, iliac artery, and tail artery were isolated and immersed in ice-cold Krebs–Henseleit (KH) solution (in mM: NaCl, 118; KCl, 4.7; CaCl\(_2\), 2.55; MgSO\(_4\), 1.18; KH\(_2\)PO\(_4\), 1.18; NaHCO\(_3\), 24.8; and glucose, 11.1). The arteries were stored overnight in KH solution at 4°C. All of the arteries were cut into ring segments around 2 mm in width. Each segment was mounted on a myograph (Multi Myograph Model 610M; Danish Myo Technology A/S, Aarhus, Denmark) with two tungsten wires (40 mm O.D.) in KH solution at 37°C aerated with 95% O\(_2\)/5% CO\(_2\). Isometric tension was measured and recorded using a data acquisition program (Myodaq 2.01; Danish Myo Technology A/S). A resting tension of 5 mN was loaded on the tail and iliac arteries, and 15 mN was loaded on the aorta. After a suitable stabilization period, the bath solution was replaced with high K\(^+\) solution (in mM: NaCl, 42.7; KCl, 80; CaCl\(_2\), 2.55; MgSO\(_4\), 1.18; KH\(_2\)PO\(_4\), 1.18; NaHCO\(_3\), 24.8; and glucose, 11.1). This procedure was repeated until stable contraction was attained. The response was evaluated by the maximal contractile force or the area under curve (AUC) from 0 to 15 min. The maximal response and 50% effective concentration (EC\(_{50}\)) were obtained by fitting the data to Hill’s equation. The endothelium was removed by scraping the lumen with silk ligature, and the removal was confirmed by a lack of relaxant response to acetylcholine (1 \(\mu\)M).

Statistical Analysis All results are expressed as the mean±standard error of the mean (S.E.M.). Statistical analysis was performed with paired t-test and Dunnett’s multiple comparison. Results with p values of less than 0.05 were considered significant.

Drugs Noradrenaline bitartrate, acetylcholine, prazosin, phenylephrine, 2-aminoethoxydiphenyl borate (2-APB), tyramine hydrochloride, and nifedipine were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.), 3,4-dihydro-6,7-
dimethoxy-α-phenyl-N,N-bis[2-(2,3,4-trimethoxyphenyl)ethyl]-1-isouquinolineacetamide hydrochloride (LOE908) was purchased from Tocris Bioscience (Ellisville, MO, U.S.A.), and ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) was purchased from Dojindo (Kumamoto, Japan).

RESULTS

High K⁺-Induced Contraction Depends on Artery Type

As shown in Fig. 1, the shape of the contraction induced by 80 mM K⁺ differed by type of artery; high K⁺ stimulation induced a slowly developing tonic contraction in the aorta and iliac artery, whereas it produced a biphasic response, i.e., a large phasic contraction followed by a tonic contraction, in the tail artery. The high K⁺-induced contraction was almost abolished by the VDCC inhibitor nifedipine (1 µM) in the aorta, and markedly inhibited in the iliac artery. In contrast, nifedipine (1 µM) only partially inhibited the high K⁺-induced contraction in the tail artery (Fig. 1). Therefore, the contribution of NSCC in the tail artery was further investigated. The

Fig. 1. Effects of Nifedipine on High K⁺-Induced Contraction in the Aorta, Iliac, and Tail Arteries

(A) Typical traces of contraction induced by 80 mM K⁺ in the absence or presence of nifedipine (1 µM) in each artery. (B) Effects of nifedipine (1 µM) on area under the curve (AUC) of contraction for the first 15 min induced by 80 mM K⁺ were summarized. Data were normalized to the control AUC without nifedipine (n=4). ** p<0.01 vs. the control.

Fig. 2. Effects of NSCC Inhibitors, SK&F96365 (10 µM), LOE908 (10 µM), and 2-APB (100 µM), on High-K⁺-Induced Contraction in the Presence of Nifedipine (1 µM) in the Tail Artery

The contraction 1 and 15 min after the application of 80 mM K⁺ in the presence of each inhibitor was normalized to that induced by 80 mM K⁺ without nifedipine (n=4). ** p<0.01 vs. the control. Dunnett’s multiple comparison was used.
NSCC inhibitors SK&F96365 (10 \( \mu \text{M} \)), LOE908 (10 \( \mu \text{M} \)), or 2-APB (100 \( \mu \text{M} \)) were applied in the presence of nifedipine (1 \( \mu \text{M} \)). Each inhibitor largely suppressed the high K\(^+\)-induced, nifedipine-insensitive contraction (Fig. 2). Interestingly, 2-APB inhibited contraction more strongly than the other two inhibitors at 1 min, while all three inhibitors produced the

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**Fig. 3. Effects of Prazosin on High K\(^+\)-Induced Contraction in the Aorta, Iliac and Tail Arteries**

(A) Typical traces of contraction induced by 80 mM K\(^+\) in the absence or presence of prazosin (0.3 \( \mu \text{M} \)). (B) Effects of prazosin (0.3 \( \mu \text{M} \)) on AUC of contraction for the first 15 min induced by 80 mM K\(^+\) were summarized. Data were normalized to the control AUC without prazosin (n=4). ** \( p < 0.01 \) vs. the control.

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**Fig. 4. Effects of the Combination of Nifedipine and Prazosin or SK&F96365 on High K\(^+\)-Induced Contraction in the Tail Artery**

(A) Typical traces of contraction induced by 80 mM K\(^+\) in the absence or presence of nifedipine (1 \( \mu \text{M} \)) plus prazosin (0.3 \( \mu \text{M} \)) or SK&F96365 (10 \( \mu \text{M} \)). (B) Effects of prazosin (0.3 \( \mu \text{M} \)) on high-K\(^+\)-induced contraction in the presence of nifedipine (1 \( \mu \text{M} \)) in the tail artery. The contraction at 1 and 15 min after the application of 80 mM K\(^+\) in the presence of each inhibitor was normalized to that induced by 80 mM K\(^+\) without nifedipine (n=4–5). ** \( p < 0.01 \) vs. the control.
In high K\(^+\) line released from the sympathetic nerve terminals is involved in the presence of 0.2 mM EGTA in the isolated arteries used.

1-adrenoceptor antagonist prazosin (0.3 µM) largely suppressed the remaining contraction in the presence of nifedipine, especially the tonic contraction, but not in the aorta, suggesting that noradrenaline released from sympathetic nerve terminals is involved in the high K\(^+\)-induced contraction in the tail and iliac arteries, but not in the aorta, suggesting that noradrenaline released from sympathetic nerve terminals is involved in the high K\(^+\)-induced contraction, depending on the type of artery. It is plausible, therefore, that high K\(^+\) stimulates not only smooth muscle cells, but also the terminals or axons of sympathetic neurons, which leads to noradrenaline release and subsequent activation of NSCCs, thereby eliciting contraction in some types of arteries. Our results also showed that tyramine elicited contraction in the iliac and tail arteries, but scarcely did so in the aorta. Nilsson et al. have reported that the density of adrenergic innervation is lower in the aorta than in the smaller arteries. Whether noradrenaline participates in high K\(^+\)-induced contraction may depend on the density of the sympathetic nerve fibers.

Tyramine induced a prominent contraction in the iliac and tail arteries, although the maximum response to tyramine was somewhat larger in the tail artery than in the iliac artery. This may explain the comparable inhibitory effects of prazosin on the high K\(^+\)-induced contraction in the iliac and tail arteries, assuming that high K\(^+\) stimulation can cause noradrenaline release from sympathetic nerve terminals in these preparations. However, there was a large difference in the degree of inhibitory effect that nifedipine had on the high K\(^+\)-induced contractions in these two kinds of arteries: It markedly inhibited contraction in the iliac artery, but only partially inhibited it in the tail artery. This may be explained by the differential contribution of VDCCs to the contraction via \(\alpha_1\)-adrenoceptors in these arteries. Our preliminary experiments showed that nifedipine largely inhibited the contraction induced by phenylephrine, an \(\alpha_1\)-adrenoceptor agonist, in the iliac artery, but it caused only a small inhibition in this type of contraction in the tail artery (Ishida H, unpublished data).

In general, arterial smooth muscle undergoes a slowly developing tonic contraction, as compared with the fast developing contraction of phasic muscle such as intestinal smooth muscle. In contrast, cutaneous arteries act like phasic muscle with a transient contraction. The present study clearly showed that in the tail artery, high K\(^+\) stimulation still caused a transient phasic contraction, followed by a sustained tonic contraction, even in the presence of prazosin. Since the tonic contraction was more sensitive to prazosin than the phasic one, the contribution of nerve-derived noradrenaline seems to be larger in the sustained tonic contraction. It may be noted that the inhibition of high K\(^+\)-induced contraction by 2-APB...
was larger than that by other NSCC inhibitors at 1 min. Since 2-APB inhibits not only NSCCs but also inositol 1,4,5-trisphosphate (IP$_3$) receptors on the sarcoplasmic reticulum, Ca$^{2+}$ release through IP$_3$ receptors may be involved in the contraction, especially in the phasic one, in the tail artery. Further experiments are required to confirm this possibility.

Prazosin or NSCC inhibitors in combination with nifedipine could not completely suppress the high K$^+$-induced contraction, whereas the chelation of extracellular Ca$^{2+}$ with EGTA completely abolished it (data not shown). Therefore, Ca$^{2+}$ influx through a pathway other than VDCCs and NSCCs activated via $\alpha_1$-adrenoceptor activation also seems to be involved. While the tonic contraction was nearly abolished by the combination of nifedipine and prazosin or the NSCC inhibitor, the phasic contraction was less sensitive to these inhibitors. One mediator that may be involved in the contraction that remained is ATP, which is co-transmitted with noradrenaline from sympathetic nerve terminals. ($^{12}$) It is plausible that released ATP increases Ca$^{2+}$ influx through NSCCs activated via P2Y receptors ($^{13,14}$) or through P2X receptor cation channels, ($^{15,16}$) thereby eliciting contraction, especially phasic contraction. Several studies have also suggested the involvement of ATP in vasoconstriction induced by sympathetic nerve stimulation. ($^{17,18}$)

In response to vasoconstrictor agonists, a slowly developing monophasic contraction is induced in splanchnic arteries such as aorta and iliac arteries, whereas biphasic contraction is induced in cutaneous arteries such as plantar, tail, and ear arteries. ($^{19-21}$) The present study also showed that the pattern of depolarization-induced contraction depends on the type of artery: High K$^+$-induced contraction was monophasic in the aorta and iliac artery and biphasic in the tail artery. It should be noted, however, that our results suggest the contribution of nerve-derived noradrenaline to high K$^+$-induced contraction in the tail artery. This high K$^+$-induced biphasic contraction might be dependent on the release of neurotransmitters due to high K$^+$ stimulation. Further investigation is needed to elucidate the reason that the contraction of the cutaneous arteries is biphasic.

In summary, the results of this study suggest that depolarization-induced contraction in rat aorta and iliac artery is mediated by Ca$^{2+}$ influx primarily through VDCCs, whereas that in the tail artery is mediated through both VDCCs and NSCCs. The results also suggest that, the activation of $\alpha_1$-adrenoceptors by nerve-derived noradrenaline and subsequent activation of NSCCs is involved in depolarization-induced contraction in the tail artery. High K$^+$-induced arterial contraction is generally considered to be evoked by the activation of VDCCs and is frequently used as a control to evaluate the activity of vasoactive substances. In experiments using isolated arterial preparations, consideration should be given to the differential contribution of neurotransmitters released from intrinsic nerve terminals to high K$^+$-induced contraction, depending on the type of artery.

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Conflict of Interest The authors declare no conflict of interest.

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