Different Effects of Verapamil on Cytosolic Ca$^{2+}$ and Contraction in Norepinephrine-Stimulated Vascular Smooth Muscle

Hideaki Karaki, Koichi Sato and Hiroshi Ozaki

Department of Veterinary Pharmacology, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Received June 23, 1990 Accepted September 28, 1990

ABSTRACT—Effects of verapamil on cytosolic Ca$^{2+}$ levels ([Ca$^{2+}]_{cyt}$) and contraction in fura-2-loaded rat aorta were examined. Norepinephrine (NE) induced a greater contraction than KCl for a given increase in [Ca$^{2+}]_{cyt}$. Cumulative addition of verapamil decreased the NE-stimulated [Ca$^{2+}]_{cyt}$ more strongly than contraction whereas verapamil decreased high K$^+$-stimulated [Ca$^{2+}]_{cyt}$ and contraction in parallel. In the presence of verapamil at a concentration needed to completely inhibit the high K$^+$-induced increments, NE induced a transient increase, followed by a small sustained increase in [Ca$^{2+}]_{cyt}$ which averaged 25% of that in the absence of verapamil. These changes were followed by a sustained contraction which averaged 60% of that in the absence of verapamil. In Ca$^{2+}$-free solution, NE induced only a transient increase in [Ca$^{2+}]_{cyt}$ whereas it induced a transient contraction, followed by a small sustained contraction. The second application of NE induced a small sustained contraction (10% of that in the presence of Ca$^{2+}$) without increasing [Ca$^{2+}]_{cyt}$. These changes were not affected by verapamil. These results suggest that verapamil inhibits NE-induced increase in [Ca$^{2+}]_{cyt}$, but not the Ca$^{2+}$-sensitization or Ca$^{2+}$-independent contraction, and this may be the reason why the NE-induced contraction is less sensitive to verapamil than that induced by high K$^+$.

Smooth muscle contraction is initiated by the increase in cytosolic free Ca$^{2+}$ levels ([Ca$^{2+}]_{cyt}$). It has been shown that high K$^+$-depolarization activates Ca$^{2+}$ influx to induce maintained contraction whereas norepinephrine (NE) releases Ca$^{2+}$ from the cellular store to induce an initial transient contraction, followed by activation of Ca$^{2+}$ influx to induce sustained contraction (1–3). Ca$^{2+}$ channel blockers have been shown to inhibit smooth muscle contraction by inhibiting Ca$^{2+}$ influx. The concentrations of these blockers needed to inhibit the NE-induced sustained contraction are higher than those needed to inhibit the high K$^+$-induced contraction, possibly because the pathways of Ca$^{2+}$ influx stimulated by high K$^+$ and NE are different (1, 2, 4, 5). Furthermore, a portion of the NE-induced sustained contraction is not inhibited by these blockers (2). These results indicate that the sustained contraction induced by NE is composed of at least two phases: Ca$^{2+}$ channel blocker-sensitive and insensitive contractions. The verapamil-insensitive component may be due to an increase in [Ca$^{2+}]_{cyt}$ that results from either Ca$^{2+}$ release (6, 7) or Ca$^{2+}$ influx which is mediated by a Ca$^{2+}$ channel blocker-insensitive pathway (8).

Recently, we have simultaneously measured [Ca$^{2+}]_{cyt}$ and contraction in isolated rat aorta
and found that the cumulative addition of NE induces a greater contraction than that due to KCl for a given increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ (9, 10). From these results, we have suggested that NE-induced sustained contraction is due not only to the increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ but also to the $\text{Ca}^{2+}$ sensitization of contractile elements. However, it has been shown that NE induces a small sustained contraction in the absence of extracellular $\text{Ca}^{2+}$ (and hence in the absence of $\text{Ca}^{2+}$ influx) (2, 11, 12). If this portion of the contraction is not dependent on the increase in $[\text{Ca}^{2+}]_{\text{cyt}}$, this may result in an apparent potentiation of NE-induced contraction at a given $[\text{Ca}^{2+}]_{\text{cyt}}$. In order to further clarify the mechanism of NE-induced sustained contraction, we examined the effects of verapamil on the $[\text{Ca}^{2+}]_{\text{cyt}}$-tension relationship in isolated vascular smooth muscle of rat aorta.

MATERIALS AND METHODS

The thoracic aorta was isolated from male Wistar rats (200–250 g) and cut into a spiral strip (2–3 mm wide and 5–6 mm length). Endothelium was removed by gently rubbing the intimal surface with a finger moistened with physiological salt solution containing: 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl$_2$, 1.0 mM MgCl$_2$, 23.8 mM NaHCO$_3$, 5.5 mM glucose and 0.01 mM ethylenediamine tetraacetic acid (EDTA). Each strip was equilibrated for 60–90 min in a physiological salt solution. High K$^+$ solution was made by substituting NaCl with equimolar KCl. The $\text{Ca}^{2+}$-free solution was made by removing CaCl$_2$ and adding 0.5 mM ethyleneglycol bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA). Each strip was equilibrated for 60–90 min in a physiological salt solution. High K$^+$ solution was made by substituting NaCl with equimolar KCl. The $\text{Ca}^{2+}$-free solution was made by removing CaCl$_2$ and adding 0.5 mM ethyleneglycol bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA). These solutions were saturated with 95% O$_2$ and 5% CO$_2$ mixture at 37°C and pH 7.4.

$[\text{Ca}^{2+}]_{\text{cyt}}$ was measured simultaneously with isometric muscle contraction (10) using a fluorescent $\text{Ca}^{2+}$ indicator, fura-2 (13). Approximately one half of the intimal surface of the fura-2-loaded muscle strip (approximately 3 mm $\times$ 3 mm area) was illuminated alternatively (48 Hz) at the excitation wavelength of 340 nm and 380 nm, and the amount of 500 nm fluorescence induced by 340 nm excitation ($F_{340}$) and that induced by 380 nm excitation ($F_{380}$) was measured using a fluorimeter (CAF-100, Japan Spectroscopic, Tokyo). One end of the muscle strip was attached to the bottom of the organ bath to minimize the movement of the smooth muscle strip. The end of the strip was attached to a strain gauge transducer to monitor isometric contractile tension. Changes in $F_{340}$ and $F_{380}$ were always monitored in order to know if the smooth muscle is loaded with enough fura-2 to overcome the changes in endogenous fluorescence and if the motion artifact interferes with the fluorescence. Muscle strips in which $F_{340}$ and $F_{380}$ did not move in opposite directions were not used. Muscle strips were treated for 7–8 min with 72.7 mM KCl, followed by a wash for 7–8 min with normal solution. This procedure was repeated three times, and the increments in muscle tension and $[\text{Ca}^{2+}]_{\text{cyt}}$ due to the third application of 72.7 mM KCl were taken as the reference (100%).

Since the dissociation constant of fura-2 for $\text{Ca}^{2+}$ in the cytoplasm may be different from that obtained in vitro (14), we did not calculate the absolute value of $[\text{Ca}^{2+}]_{\text{cyt}}$. Instead, we used the ratio of $F_{340}$ to $F_{380}$ as an indicator of $[\text{Ca}^{2+}]_{\text{cyt}}$ (10). In each experiment, 72.7 mM K$^+$ was applied before the experimental procedures, and the sustained level of the high K$^+$-stimulated $[\text{Ca}^{2+}]_{\text{cyt}}$ was taken as 100%. In order to use the fluorescence ratio as a relative $[\text{Ca}^{2+}]_{\text{cyt}}$, it is necessary to confirm if the ratio is in the linear portion of the calibration curve. This was confirmed by measuring the maximum and minimum fluorescence levels using 10 μM ionomycin and 4 mM EGTA, respectively, at the end of each experiment (15). Autofluorescence of the muscle was measured by applying 4 mM MnCl$_2$ and this was 30–50% of the total fluorescence in the fura-2-loaded aorta, as reported previously (10).

The following chemicals were used: verapamil hydrochloride (Sigma Chemicals, St. Louis, MO), (l)-norepinephrine bitartrate (Wako Pure Chemicals, Tokyo), ionomycin
RESULTS

Figure 1 shows the effects of high K+ and NE on contractile tension and 

\[ [Ca^{2+}]_{cyt} \]

in rat aorta. Both 72.7 mM KCl and 1 \( \mu \)M NE induced sustained increases in tension and \( [Ca^{2+}]_{cyt} \), as shown previously (10). In the presence of 10 \( \mu \)M verapamil, NE induced a transient increase in \( [Ca^{2+}]_{cyt} \) that was followed by a small sustained increments averaging 25 \( \pm \) 2.0\% \( (n = 4) \) of the \( [Ca^{2+}]_{cyt} \) obtained in the absence of verapamil. Muscle tension also showed a rapid increase, followed by a small decrease to a level that was 60.5 \( \pm \) 5.5\% \( (n = 4) \) of the contraction obtained in the absence of verapamil. Addition of 4 mM EGTA rapidly decreased \( [Ca^{2+}]_{cyt} \) to a level identical to that obtained in the presence of 4 mM EGTA and 10 \( \mu \)M ionomycin. Muscle contraction was also inhibited, although a small portion of the contraction was resistant to Ca\(^{2+}\) removal. In the presence of 10 \( \mu \)M verapamil, 72.7 mM KCl changed neither \( [Ca^{2+}]_{cyt} \) nor muscle tension \( (n = 4) \).

Figure 2 shows the effect of NE on \( [Ca^{2+}]_{cyt} \) and muscle tension in Ca\(^{2+}\)-free solution. In Ca\(^{2+}\)-free solution, 1 \( \mu \)M NE induced a transient increase in \( [Ca^{2+}]_{cyt} \), followed by a decrease to a level identical to that obtained in the presence of 10 \( \mu \)M ionomycin and 4 mM EGTA. In contrast to this, NE induced a transient contraction followed by a sustained contraction which represented approximately 10\% of the sustained contraction obtained in the presence of 1.5 mM Ca\(^{2+}\). This result is consistent with the previous observation (10, 12). After removing NE, the second application of NE did not induce the initial transient changes in muscle tension or \( [Ca^{2+}]_{cyt} \). However, it induced a sustained contraction of similar magnitude as

![Figure 1](image-url)
that of the first sustained contraction without changing the \([\text{Ca}^{2+}]_{\text{cyt}}\). High K\(^+\) changed neither muscle tension nor \([\text{Ca}^{2+}]_{\text{cyt}}\) in Ca\(^{2+}\)-free solution (n = 4). Verapamil (10 \(\mu\)M) inhibited neither the NE-induced transient increase in \([\text{Ca}^{2+}]_{\text{cyt}}\) nor the transient and sustained contractions in Ca\(^{2+}\)-free solution (n = 4).

Cumulative addition of KCl or NE increased muscle tension and \([\text{Ca}^{2+}]_{\text{cyt}}\) in rat aorta. The \([\text{Ca}^{2+}]_{\text{cyt}}\)-tension relationships in the presence of KCl and NE are shown in Fig. 3. These figures indicate that there is a positive correlation between these two parameters and that NE produced more contraction at each level of \([\text{Ca}^{2+}]_{\text{cyt}}\) than was the case for high K\(^+\), as reported previously (9, 10).

Figure 3 (left) also shows that increments in \([\text{Ca}^{2+}]_{\text{cyt}}\) and tension due to cumulative addition of KCl were inhibited in the presence of 100 nM verapamil. Since verapamil inhibited \([\text{Ca}^{2+}]_{\text{cyt}}\) and tension to a similar extent, the slope of the \([\text{Ca}^{2+}]_{\text{cyt}}\)-tension relationship did not change. Higher concentrations (1–10 \(\mu\)M) of verapamil more strongly inhibited both high K\(^+\)-induced changes without changing the slope of the \([\text{Ca}^{2+}]_{\text{cyt}}\)-tension relationship.

As shown in Fig. 3 (right), the concentrations of verapamil needed to inhibit the NE-induced changes were higher than those required to inhibit the high K\(^+\)-induced changes. Verapamil (1 \(\mu\)M) inhibited the NE-stimulated \([\text{Ca}^{2+}]_{\text{cyt}}\) and contraction with little change in the slope of the \([\text{Ca}^{2+}]_{\text{cyt}}\)-tension relationship. A higher concentration of verapamil (10 \(\mu\)M) inhibited \([\text{Ca}^{2+}]_{\text{cyt}}\) more strongly than contraction, resulting in an increase in the slope of the \([\text{Ca}^{2+}]_{\text{cyt}}\)-tension relationship. This result indicates that NE induces much greater contraction in the presence of 10 \(\mu\)M verapamil for a given increase in \([\text{Ca}^{2+}]_{\text{cyt}}\) than in the absence of verapamil. Since NE induces a contraction without increasing \([\text{Ca}^{2+}]_{\text{cyt}}\) (Figs. 1 and 2) and since verapamil does not inhibit this portion of the contraction, the augmentation of NE-induced contraction at each level of \([\text{Ca}^{2+}]_{\text{cyt}}\) may be due to a Ca\(^{2+}\)-independent portion of the contraction. In order to examine this possibility, the following experiment was performed.

Sequential addition of 100 nM, 1 \(\mu\)M and 10 \(\mu\)M verapamil during the 72.7 mM KCl-induced sustained contraction resulted in a graded decrease in \([\text{Ca}^{2+}]_{\text{cyt}}\) and muscle contraction to their respective resting levels (Fig. 4), and the slope of the resulting \([\text{Ca}^{2+}]_{\text{cyt}}\)-tension relationship was almost the same as that obtained by the cumulative addition of KCl in Fig. 3 (left). Addition of 4 mM EGTA further decreased \([\text{Ca}^{2+}]_{\text{cyt}}\) with little change in muscle tension. Figure 4 also shows that se-

---

**Fig. 2.** Effects of norepinephrine on \([\text{Ca}^{2+}]_{\text{cyt}}\) (upper trace) and contraction (lower trace) in rat aorta in Ca\(^{2+}\)-free solution. \([\text{Ca}^{2+}]_{\text{cyt}}\) is shown by the fura-2 fluorescence ratio, taking the high K\(^+\)-stimulated level as 100%. Muscle was stimulated by 72.7 mM KCl (K) in the presence of 1.5 mM Ca\(^{2+}\) and then washed with Ca\(^{2+}\)-free solution containing 0.5 mM EGTA (0 mM Ca). After the \([\text{Ca}^{2+}]_{\text{cyt}}\) decreased to the resting level, 1 \(\mu\)M norepinephrine (NE) was added. After washing the muscle with Ca\(^{2+}\)-free solution, norepinephrine was added again.
Verapamil on Aortic Contraction and $[\text{Ca}^{2+}]_{\text{cyt}}$

Fig. 3. $[\text{Ca}^{2+}]_{\text{cyt}}$-tension relationship obtained in rat aorta by cumulative addition of KCl (left) or nor-epinephrine (right) in the absence (control) or presence of verapamil. Muscle contraction (ordinate) and increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ (abscissa) induced by previous application of 72.7 mM KCl were used as the standard (100%), and then norepinephrine or KCl was cumulatively added. Verapamil was added 5 min before the cumulative addition of the stimulants. Numbers indicate the concentration of KCl (1: 9.8 mM, 2: 14.2 mM, 3: 24.2 mM, 4: 38.7 mM, 5: 78.0 mM) or norepinephrine (1: 3 nM, 2: 10 nM, 3: 30 nM, 4: 100 nM, 5: 300 nM, 6: 1 μM). Mean ± S.E. of 4 to 6 experiments are shown.

Fig. 4. $[\text{Ca}^{2+}]_{\text{cyt}}$-tension relationship obtained in rat aorta in the presence of norepinephrine (■: 1 μM, △: 100 nM) and KCl (●: 72.7 mM). Muscle contraction (ordinate) and increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ (abscissa) induced by previous application of 72.7 mM KCl were used as the standard (100%). Washing the muscle with normal solution, norepinephrine or KCl was added to induce a sustained contraction. Verapamil and EGTA were then cumulatively added to decrease the muscle tension and $[\text{Ca}^{2+}]_{\text{cyt}}$. Numbers indicate the results in the absence of verapamil or EGTA (1) or in the presence of 100 nM verapamil (2), 1 μM verapamil (3), 10 μM verapamil (4) and 4 mM EGTA (5). The mean ± S.E. of 4 to 6 experiments is shown for each condition.
Sequential addition of verapamil and EGTA during the NE (100 nM or 1 μM)-induced sustained contraction resulted in a graded decrease in [Ca^{2+}]_cyt and muscle contraction. However, since verapamil inhibited [Ca^{2+}]_cyt more strongly than muscle tension, the [Ca^{2+}]_cyt-tension relationship due to 100 nM NE shifted to the lower [Ca^{2+}]_cyt levels in parallel to the curve obtained in the presence of 72.7 mM KCl. In the presence of a higher concentration (1 μM) of NE, the curve was further shifted to the left, indicating that the effect of NE to shift the [Ca^{2+}]_cyt-tension relationship is concentration-dependent. The Ca^{2+}-tension relationship also suggests that muscle tension induced by 100 nM or 1 μM NE is approximately 22% and 40%, respectively, greater than that induced by high K^+ when [Ca^{2+}]_cyt is greater than the resting level. In the presence of EGTA, [Ca^{2+}]_cyt decreased to a level below the resting [Ca^{2+}]_cyt, although the small sustained contraction (6% in the presence of 100 nM NE and 8% in the presence of 1 μM NE) was not inhibited.

**DISCUSSION**

We have previously reported that NE induced sustained increases in [Ca^{2+}]_cyt and muscle tension in the presence of external Ca^{2+}, whereas it induced only transient increments in [Ca^{2+}]_cyt and tension in the absence of external Ca^{2+} (10, 12). The second application of NE did not induce the transient increments in Ca^{2+}-free solution (Fig. 2). It was also found that verapamil did not inhibit the NE-induced transient increase in [Ca^{2+}]_cyt. These results support the suggestion that the NE-induced initial transient contraction is due to the release of a limited amount of stored Ca^{2+} by a verapamil-insensitive mechanism (2, 6–8, 10).

In a Ca^{2+}-free solution, the NE-induced contraction decreased to a level slightly above the resting level, whereas the [Ca^{2+}]_cyt decreased to a level below the resting [Ca^{2+}]_cyt (Figs. 1 and 2). Although we did not calculate the absolute [Ca^{2+}]_cyt value in the present experiments, the remaining [Ca^{2+}]_cyt may be approximately 1 – 10 nM because [Ca^{2+}]_cyt decreased to a level identical to that in the presence of 4 mM EGTA and a Ca^{2+} ionophore 10 μM ionomycin, which may abolish the Ca^{2+} gradient across the plasma membrane. These results support the suggestion (12) that the NE-induced small sustained contraction obtained in a Ca^{2+}-free solution is attributable to a Ca^{2+}-independent mechanism.

Verapamil inhibited high K^+-stimulated [Ca^{2+}]_cyt and muscle tension without changing the slope of [Ca^{2+}]_cyt-tension relationship constructed by either the cumulative addition of KCl (Fig. 3) or the cumulative addition of verapamil (Fig. 4). These results suggest that the contractile effect of KCl is attributable to the increase in [Ca^{2+}]_cyt and that the inhibitory effect of verapamil is due to the decrease in [Ca^{2+}]_cyt. Verapamil also inhibited the NE-induced sustained increase in [Ca^{2+}]_cyt, suggesting that NE opens the verapamil-sensitive Ca^{2+} influx pathway. The fact that the concentration of verapamil required to inhibit the NE-stimulated [Ca^{2+}]_cyt was 10 times higher than that needed to inhibit the high K^+-stimulated [Ca^{2+}]_cyt supports the suggestion that the pathways of Ca^{2+} influx activated by NE and high K^+ are different (1–4).

Verapamil more strongly inhibited the NE-stimulated [Ca^{2+}]_cyt than contraction, resulting in a dissociation between muscle tension and [Ca^{2+}]_cyt. Previously, it has been suggested that a part of the NE-induced contraction is not inhibited by verapamil because a part of the NE-stimulated Ca^{2+} influx is not sensitive to verapamil (e.g., ref. 10). The present results suggest that the verapamil-insensitive portion of the NE-induced contraction is not solely due to the inability of verapamil to inhibit the NE-stimulated Ca^{2+} influx. Verapamil increased the slope of the [Ca^{2+}]_cyt-tension relationship constructed by the cumulative addition of NE (Fig. 3). In order to know if the Ca^{2+}-independent portion of the contraction is responsible for the increase in the slope of the [Ca^{2+}]_cyt-tension relationship, a different experimental procedure was em-
ployed. Sequential addition of verapamil during the sustained contraction induced by a fixed concentration of NE (Fig. 4) showed that NE induced a concentration-dependent shift of the curve to the lower $[\text{Ca}^{2+}]_{\text{cyt}}$ levels in parallel to that obtained in the presence of KCl. Addition of EGTA further decreased $[\text{Ca}^{2+}]_{\text{cyt}}$, although 6–8% of the NE-induced contraction was not inhibited. Since the 100 nM or 1 mM NE-induced contraction at a given $[\text{Ca}^{2+}]_{\text{cyt}}$ was greater than that induced by high K+ by 22% or 40%, respectively, the shift of the curve is not solely due to the Ca$^{2+}$-independent contraction (6–8%). These results suggest that the NE-induced sustained contraction is attributable not only to the increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ but also to Ca$^{2+}$ sensitization and a Ca$^{2+}$-independent mechanism.

In vascular smooth muscle, it has been reported that endothelin also shifts the $[\text{Ca}^{2+}]_{\text{cyt}}$-tension relationship to lower $[\text{Ca}^{2+}]_{\text{cyt}}$ levels (16). Furthermore, the activators of protein kinase C, phorbol esters, show similar effects on the $[\text{Ca}^{2+}]_{\text{cyt}}$-tension relationship (17, 18). Recently, it has been shown in the bacterial α-toxin permeabilized smooth muscle that NE (19), phenylephrine (20) and phorbol ester (21) increase the Ca$^{2+}$ sensitivity of contractile elements and that an inhibitor of protein kinase C inhibits the Ca$^{2+}$ sensitizing effect (19). These results suggest that the activation of protein kinase C by diacylglycerol, which is produced by the receptor-mediated breakdown of phosphoinositides, might be involved in the Ca$^{2+}$ sensitization in permeabilized smooth muscle preparations. A similar mechanism may be responsible for the Ca$^{2+}$ sensitization in the intact smooth muscle tissue (12, 14). Although the biochemical background for Ca$^{2+}$ sensitization is not clarified as yet, it has been suggested that the inhibitory effect of calponin on the actin-myosin interaction (22) is decreased by phosphorylation of calponin by protein kinase C (23).

In conclusion, it is suggested that the NE-induced sustained contraction is attributable to the increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ mediated by verapamil-sensitive and insensitive Ca$^{2+}$ influx pathways, Ca$^{2+}$ sensitization, and a Ca$^{2+}$-independent mechanism. Verapamil inhibits the NE-induced increase in $[\text{Ca}^{2+}]_{\text{cyt}}$, but not Ca$^{2+}$ sensitization or Ca$^{2+}$-independent contraction, and this may be the reason why the NE-induced contraction is less sensitive to verapamil than that induced by high K$^+$. 

Acknowledgments
We are grateful to Dr. Tatsuya Ohyama of our department for his help. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1 Bolton, T.B.: Mechanism of action of transmitters and other substances on smooth muscle. Physiol. Rev. 59, 606–718 (1979)
2 Karaki, H. and Weiss, G.B.: Calcium channels in smooth muscle. Gastroenterology 87, 960–970 (1984)
3 Karaki, H. and Weiss, G.B.: Calcium release in smooth muscle. Life Sci. 42, 111–122 (1988)
4 Van Breemen, C., Aaronson, P. and Loutzenhiser, R.: Sodium-calcium interactions in mammalian smooth muscle. Pharmacol. Rev. 30, 167–208 (1979)
5 Flaim, S.F.: Comparative pharmacology of calcium blockers based on studies of vascular smooth muscle. In Calcium Blockers, Edited by Flaim, S.F. and Zelis, R., p. 155–178, Urban & Schwarzenberg, New York (1982)
6 Bou, J., Llenas, J. and Massingham, R.: Calcium entry blocking drugs, "calcium antagonists" and vascular smooth muscle function. J. Auton. Pharmacol. 3, 219–232 (1983)
7 Spedding, M. and Cavero, I.: “Calcium antagonists”: a class of drugs with a bright future. Part II. Determination of basic pharmacological properties. Life Sci. 35, 575–587 (1984)
8 Cauvin, C., Loutzenhiser, R. and Van Breemen, C.: Mechanism of calcium antagonists-induced vasodilation. Amnu. Rev. Pharmacol. Toxicol. 23, 373–396 (1983)
9 Karaki, H., Sato, K. and Ozaki, H.: Different effects of norepinephrine and KCl on cytosolic Ca$^{2+}$-tension relationship in vascular smooth muscle of rat aorta. Eur. J. Pharmacol. 151, 325–328 (1988)
10 Sato, K., Ozaki, H. and Karaki, H.: Changes in cytosolic calcium level in vascular smooth muscle
strip measured simultaneously with contraction using fluorescent calcium indicator fura 2. J. Pharmacol. Exp. Ther. 246, 294–300 (1988)

11 Heaslip, R.J. and Rahwan, R.G.: Evidence for the existence of two distinct pools of intracellular calcium in the rat aorta accessible to mobilization by norepinephrine. J. Pharmacol. Exp. Ther. 221, 7–13 (1982)

12 Ozaki, H., Ohyama, T., Sato, K. and Karaki, H.: Ca\(^{2+}\)-dependent and independent mechanisms of sustained contraction in vascular smooth muscle of rat aorta. J. Pharmacol. 52, 509–512 (1982)

13 Grynkiewicz, G., Poenie, M. and Tsien, R.Y.: A new generation of Ca\(^{2+}\) indicators with greatly improved fluorescence properties. J. Biol. Chem. 260, 3440–3450 (1985)

14 Karaki, H.: Ca\(^{2+}\) localization and sensitivity in vascular smooth muscle. Trends Pharmacol. Sci. 10, 320–325 (1989)

15 Mitsui, M., Nakao, K., Inukai, T. and Karaki, H.: Inhibitory effects of cadirolazine and its metabolite, ISF-240S, on contractions and the level of cytosolic Ca\(^{2+}\) in vascular smooth muscle. Eur. J. Pharmacol. 178, 171–177 (1990)

16 Sakata, K., Ozaki, H., Kwon, S.-C. and Karaki, H.: Effects of endothelin on the mechanical activity and cytosolic calcium levels of various types of smooth muscle. Br. J. Pharmacol. 98, 483–492 (1989)

17 Karaki, H., Ozaki, H., Ohyama, T., Sato, K. and Kato, N.: Protein kinase C mediates the contraction induced by receptor agonists in vascular smooth muscle. Japan. J. Pharmacol. 49, Supp. 96P (1989)

18 Sato, K., Ozaki, H. and Karaki, H.: Inhibitory effect of verapamil on cytoplasmic Ca level and muscle tension in vascular smooth muscle. Japan. J. Pharmacol. 49, Supp. 97P (1989)

19 Nishimura, J., Kolber, M. and Van Breemen, C.: Norepinephrine and GTP-γ-S increase myofilament Ca\(^{2+}\) sensitivity in α-toxin permeabilized arterial smooth muscle. Biochem. Biophys. Res. Commun. 157, 677–683 (1988)

20 Kitazawa, T., Kobayashi, S., Horiiuti, K., Somlyo, A.V. and Somlyo, A.P.: Receptor-coupled, permeabilized smooth muscle. J. Biol. Chem. 264, 5324–5339 (1989)

21 Nishimura, J. and Van Breemen, C.: Direct regulation of smooth muscle contractile filaments by second messengers. Biochem. Biophys. Res. Commun. 163, 929–935 (1989)

22 Takahashi, K., Hiwada, K. and Kokubu, T.: Isolation and characterization of 3400-dalton calmodulin- and actin-binding protein from chicken gizzard smooth muscle. Biochem. Biophys. Res. commun. 141, 20–26 (1986)

23 Winder, S.J. and Walsh, M.P.: Smooth muscle calponin, inhibition of actomyosin Mg ATPase and regulation by phosphorylation. J. Biol. Chem. 265, 10148–10155 (1990)