Comparison of Contractile Effects of Sodium Vanadate and Ouabain in Vascular Smooth Muscles of Guinea-pigs and rats

Tomoko SHIMADA, Akio TSUJI, Keiichi SHIMAMURA
and Satoru SUNANO

Research Institute of Hypertension, Kinki University, Sayama-cho, Osaka, Japan

Abstract

Shimada, T., Tsuji, A., Shimamura, K. and Sunano, S. *Comparison of contractile effects of sodium vanadate and ouabain in vascular smooth muscle of guinea-pig and rats.* Japanese Journal of Smooth Muscle Research, 1986, 22(5); 409-422 — Ouabain and vanadate are known as potent inhibitors of Na, K-ATPase in various tissues including smooth muscles. Both agents showed contractile action on various smooth muscles in a similar fashion: stronger contractile action on the aortae of rats (WKY and stroke prone spontaneously hypertensive rats, SHRSP) and guinea-pigs, and weaker contractile actions on basilar and mesenteric arteries of the same animals. Time to peak tension, however, was far longer in ouabain-induced contraction. Phentolamine depressed ouabain-induced contractions, while vanadate-induced contractions were not affected. Elevation of K^+ concentration to 20 or 30 mM potentiated vanadate-induced contraction markedly, while it potentiated ouabain-induced contraction only slightly. DIDS blocked vanadate-induced contraction but showed no effect on ouabain-induced contraction. Removal of Ca abolished ouabain-induced contractions, while vanadate-induced contractions of reduced height could be observed in the absence of Ca. Verapamil depressed both ouabain- and vanadate-induced contractions of WKY and SHRSP aorta but exhibited no effect on the guinea-pig aorta. Thus, although similarities of the action of ouabain and sodium vanadate were observed, the modes of the actions were revealed to be different in the two agents. Inhibition of Na, K-ATPase might be involved in the case of ouabain-induced contractions, and inhibition of Ca-ATPase of membranous systems might be involved in vanadate-induced contraction.

Key words: Vanadate, Ouabain, Na, K-ATPase, Vascular Smooth Muscles.

Introduction

Vanadate has been recognized as a potent inhibitor of Na, K-ATPase, since it was found in muscle (Josephson and Cantley, 1977; Cantley *et al.*, 1977). It inhibits Na, K-ATPase or Na, K-pump of smooth muscles, as has been demonstrated directly or indirectly (Grover *et al.*, 1980; Ueda *et al.*, 1982; Searle *et al.*, 1983; Ueda and Urakawa, 1983). The inhibition of Na,

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嶋田朋子, 辻 章夫, 島村佳一, 砂野 哲: 近畿大学高血圧研究所, 〒589 大阪府南河内郡狭山町西山 380

K-ATPase induces contractions of vascular smooth muscles, as has been reported mostly in experiments which were performed using ouabain treatment (Broekart and Godfraind, 1973; Karaki and Urakawa, 1977; Karaki *et al.*, 1978; Ozaki *et al.*, 1978; Toda, 1980; Palaty, 1980; Nilson and Mulvany, 1981; Moreland *et al.*, 1984; Ishida *et al.*, 1985). In these reports, it was revealed that the efficiency of ouabain in initiating contraction varies among types of vascular smooth muscles. In addition, it has been reported that ouabain at concentrations up to 10^{-2} M did not cause contraction in rat resistance vessels (Mulvany *et al.*, 1982; Mulvany *et al.*, 1984; Aalkjaer and Mulvany, 1985). Modes of ouabain action on vascular smooth muscles are also different among types of vascular tissues: direct action on vascular smooth muscles and indirect action through the release of intrinsic neurotransmitters, roles of Ca in the initiation of contraction, etc.

Vanadate has also been reported to induce contractions of vascular smooth muscles (Huot *et al.*, 1979; Ozaki and Urakawa, 1980; Rapp, 1981). We have recently reported that the efficiency of vanadate in the initiation of contraction varied among types of vascular smooth muscles (Shimada *et al.*, 1986). These variations seemed to be similar to those in the action of ouabain mentioned above.

Therefore, the present experiments were performed to compare the effects of vanadate and ouabain and also some modes of their actions in vascular smooth muscles of guinea-pigs and rats.

Methods

Guinea-pigs (519 ± 20.3 g, mean $\pm$ SE, $n=18$), Wistar Kyoto rats (WKY, 249 ± 4.1 g, mean $\pm$ SE, $n=12$) and stroke prone spontaneously hypertensive rats (SHRSP, 245 ± 7.7 g, mean $\pm$ SE, $n=21$) were used in the present experiments. The systolic blood pressures of WKY and SHRSP, measured by the tail cuff method, were 133 ± 2.5 mmHg (mean $\pm$ SE, $n=12$) and 216 ± 4.5 mmHg (mean $\pm$ SE, $n=16$), respectively. Animals were anaesthetized with ether and killed by bleeding from the carotid artery. Aortae, mesenteric arteries of all animals and basilar arteries of guinea-pigs were dissected, and fat and connective tissues were removed carefully in modified Tyrode's solution. From these aortae and arteries, spiral cut preparations were made under a dissecting microscope. The widths of the aortae, mesenteric artery and basilar artery preparations were about 1.0 mm, 0.2 mm and 0.1 mm, respectively. These preparations were incubated in organ baths of 10 ml filled with modified Tyrode's solution of the following composition: NaCl, 137 mM; KCl, 2.7 mM; CaCl_2 , 2.0 mM; MgCl_2 , 1.0 mM; NaHCO_3 , 11.9 mM; NaH_2PO_4 , 0.4 mM; glucose, 5.6 mM, equilibrated with a gas mixture of 95% O_2 and 5% CO_2 . K-Tyrode's solution was made by replacing all NaCl of modified Tyrode's solution with KCl, and elevated K-Tyrode's solutions were made by mixing the modified and K-Tyrode's solution to obtain the desired K-concentrations. Ca-free Tyrode's solution was made by omitting CaCl_2 of the modified Tyrode's solution. No correction of changes in osmolarity due to the omission of CaCl_2 was made, since no obvious effect on contractile responses was observed by similar changes in osmolarity made by the reduction of NaCl (Sunano, unpublished observation). The temperature of the solution was kept at 36°C by circulating water of the same temperature in the outer chamber of the organ bath. The temperature of circulating

water was controlled by a thermoregulator (Yamato, Tokyo). The pH of the modified Tyrode's solution at this temperature was between 7.2 and 7.3.

Changes in tension were measured isometrically by force displacement transducers (Shinkoh, U-gage, Tokyo) and recorded by a thermal-pen recorder (San-ei Sokki).

The experiments were always started from the repetition of K-contraction, which was induced by changing the incubation medium from the modified Tyrode's solution to K-Tyrode's solution. The durations and interval of the application of K-Tyrode's solution varied among the preparations since the time to peak tension and relaxation time varied. The contraction heights increased by the repetition and achieved maximum by 4 to 5 times repetition. The height of the fifth K-contraction was treated as the control K-contraction height, and most of the drug-induced contractions were expressed as percentages of the contraction heights of the respective preparations.

Drugs used in the present experiments were: sodium metavanadate (NaVO_3 , Wako, Osaka), ouabain (Sigma), phentolamine (Ciba), 4, 4'-diisothiocyano-2, 2'-disulfonic acid stilbene (DIDS, Wako, Osaka), and verapamil (Ei-sai).

For statistical evaluation, Student's t-test was employed and was considered significant when p value was smaller than 0.05.

Results

1. Comparison of contractile actions of sodium vanadate and ouabain in various vascular smooth muscles.

Fig. 1 shows the contractile responses of the aorta, mesenteric artery and basilar artery of guinea-pig to 1 mM sodium vanadate. This concentration (10^{-3} M) was chosen because it was just submaximal in most of the preparations, regardless of the variation of the maximum response (Shimada *et al.*, 1986). As shown in this figure, the highest contractile response was observed in the aorta, but it was still lower than the height of K-contraction. Both in the mesenteric and basilar arteries, the contractile responses to sodium vanadate were extremely low as compared with those to K-Tyrode's solution (K-contraction). By washing out vanadate with modified Tyrode's solution, the developed tension returned to the initial level within 10 to

Fig. 1. Effects of sodium vanadate on various vascular smooth muscles of the guinea-pig. a, m and b indicate aorta, mesenteric artery and basilar artery, respectively. Sodium vanadate (NaVO_3 , 10^{-3} M) was applied after the observation of K-Tyrode's solution (K-T)-induced contraction. Downward arrows indicate the washing out with modified Tyrode's solution.

Fig. 2. Ouabain-induced tension developments in various vascular smooth muscles of the guinea-pig. Ouabain of 10^{-4} M (Ouab) was applied in this experiment. K-T, the application of K-Tyrodé's solution. a, m and b indicate the same as those in Fig. 1.

20 minutes.

Similar results were obtained in the aortae and mesenteric arteries of WKY and SHRSP. The rates of tension development and peak tensions of aortae of these animals were significantly greater than those of the guinea-pig aorta.

Contractile effects of ouabain were also studied. As shown in Fig. 2, 10^{-4} M ouabain induced marked tension development which is comparable to or higher than that of K-contracture in the guinea-pig aorta. In the mesenteric and basilar artery, on the other hand, no obvious tension development could be observed up to 120 minutes of application in most of the preparations. When the application period was prolonged further, gradual tension developments, which achieved their maxima at 180 to 200 minutes after the application, could be observed. However, the developed tensions were far smaller when compared with those of K-contracture. The recovery process after the washing out of ouabain were also slow, and at least 120 minutes were required to achieve complete relaxation.

In aortae of WKY and SHRSP, ouabain of 10^{-3} M, which was 10 times higher than the concentration used in the guinea-pigs, induced initial small tension development followed by a second elevation of tension. The second contractions achieved their maxima at 90 to 120 minutes after the application of ouabain, and they were smaller than those of K-contracture. In the mesenteric artery of WKY, no tension development could be observed even 300 minutes after the application of 10^{-3} M ouabain, while weak but significant tension development could be observed in the mesenteric artery of SHRSP. These comparative results were summarized in Fig. 3 and Table 1.

As shown in Fig. 3, the contractile response to sodium vanadate and ouabain varied among the types of blood vessels in a similar fashion: it was larger in the aortae and smaller in the other blood vessels. When both contractions were compared, vanadate-induced contractions were greater in WKY and SHRSP blood vessels and smaller in guinea-pig blood vessels. It should also be mentioned that in WKY and SHRSP blood vessels, ouabain of 10 times higher concentration than that used in the guinea-pig blood vessels was used in the present experiments.

A greater difference in the action of vanadate and ouabain was observed in the time to peak

Fig. 3. Heights of sodium vanadate- and ouabain-induced contractions in various vascular smooth muscles of different animals. Control K-Tyrod's-induced contracture heights were taken as 100 percent, and each contraction height was expressed as percentages of these heights with SEs (vertical bars). Numbers at the top of each column indicate the number of respective experiment. Sodium vanadate (NaVO₃) of 10⁻³ M in all animal species, ouabain of 10⁻³ M in SHRSP and WKY and 10⁻⁴ M in the guinea-pig, were, respectively, used. a, m and b indicate the same as Fig. 1.

tension (Table 1). Vanadate-induced contractions attained their maxima within 30 minutes after the application in the majority of preparations, whereas at least 100 minutes were required to achieve the peak in ouabain-induced contraction. In the mesenteric artery of SHRSP, for example, it took 240 minutes to reach peak tension, in contrast to 37 minutes in the case of vanadate-induced contraction. Moreover, no tension development was observed in the mesenteric artery of WKY within 300 minutes after the application of ouabain, as described above.

Effects of phentolamine on ouabain- and sodium vanadate-induced contraction were studied since the involvements of noradrenaline release in the ouabain-induced contraction have been presented, as will be mentioned in the discussion. Aortae of WKY, SHRSP and guinea-pigs were used in these experiments, as they showed greater contractile responses to both ouabain and sodium vanadate. In these aortae, phentolamine of 5×10^{-5} M completely blocked 10⁻⁶ M noradrenaline-induced contraction. However, phentolamine of the same concentration showed no effect on sodium vanadate-induced contraction of all aortae (Fig. 4). Ouabain-induced contractions, on the other hand, were significantly depressed by the treatment with phentolamine of the same concentration (Fig. 4).

Table 1. Time to peak tension in vanadate- and ouabain-induced contractions.

	Time to peak tension (minutes)	
	Vanadate	Ouabain
	aorta	
SHRSP	21±3.1(7)	105 ± 11.1(7)
WKY	23±1.9(6)	103 ± 4.3(6)
Guinea-pig	34±1.8(6)	105 ± 5.4(6)
	mesenteric artery	
SHRSP	37±5.2(6)	240 ± 7.0(6)
WKY	25±0.9(6)	— (6)
Guinea-pig	25±2.7(7)	186 ± 10.6(7)

Each value expresses mean±SE of the results of numbers shown in parenthesis.

Fig. 4. Heights of sodium vanadate- and ouabain-induced contractions of aortae in the presence of phentolamine. Heights of respective K-contraction were taken as 100 percent, and each contraction height was expressed as a percentage of this height with SEs (vertical bars). Phentolamine of 5×10^{-5} M was applied 10 minutes before the application of either drug. Sodium vanadate (NaVO_3) of 10^{-3} M was used in all the preparations. Concentrations of ouabain used were 10^{-3} M in SHRSP and WKY aortae and 10^{-4} M in guinea-pig aortae. Open columns, control sodium vanadate- or ouabain-induced contractions. Stippled columns, contraction heights in the presence of phentolamine. Numbers at the top of each column indicate the number of respective experiment. Asterisks indicate significant differences from control ($p < 0.001$).

2. Effects of increased K-concentration and DIDS.

Vanadate-induced contractions of mesenteric arteries of all animal species were extremely small, as described above. The vanadate-induced contractions of mesenteric arteries were potentiated markedly in the presence of 20 to 30 mM K^+ (Figs. 5 and 6). In the mesenteric artery of SHRSP shown in Fig. 5, 20 mM K caused slight and 30 mM K caused more marked increase in the basal tension. The addition of 1 mM sodium vanadate in the presence of elevated K^+ caused slow but marked tension development up to about 60% of the height of K-contraction.

Ouabain induced no tension development in the mesenteric artery of SHRSP within the application period of 30 minutes (Fig. 5). In the presence of 20 mM K^+ , it induced a quick elevation of tension which was sustained for at least 30 minutes. Ouabain-induced tension development was more potentiated in the presence of 30 mM K^+ . However, it became transient and relaxed toward the level before the application. The potentiation of sodium vanadate- and ouabain-induced contraction could also be observed in the guinea-pig mesenteric artery, as summarized in Fig. 6.

Thus, although the elevation of K^+ concentration to 20 or 30 mM potentiated both sodium vanadate- and ouabain-induced contractions, the mode of and the degree of the potentiation were different between these two contractions.

In the depolarized preparation of the guinea-pig mesenteric artery soaked in K-Tyrode's solution, sodium vanadate showed dual action which was composed of the first transient contractile and the second marked relaxing actions (Fig. 7). Thus almost complete relaxation was brought about by 10^{-3} M sodium vanadate. Ouabain (10^{-4} M), on the other hand, showed

no relaxing but sustained contractile action on the K-Tyrode's solution depolarized mesenteric artery of the guinea-pig (Fig. 7). In WKY and SHRSP mesenteric arteries, no relaxing but only contractile response was observed by the application of the same concentration of sodium vanadate, as has previously been reported (Shimada *et al.*, 1986).

Aortae of all animal species also showed relaxing response to sodium vanadate preceded by initial transient contractile response. Unlike its action in the guinea-pig mesenteric artery,

Fig. 5. Effects of elevation of K^+ concentration on sodium vanadate- and ouabain-induced contractions in SHRSP mesenteric artery. K^+ concentration was elevated to 20 mM (K20) or to 30 mM (K30) 21 minutes before the application of sodium vanadate (10^{-3} M, upper figures) or ouabain (10^{-3} M, lower figures).

Fig. 6. Heights of sodium vanadate- and ouabain-induced contractions at different concentrations of K^+ . K2.7, K20 and K30 indicate K^+ concentrations of 2.7 mM, 20 mM and 30 mM, respectively. Control K-contraction height was taken as 100 percent, and each height was expressed as a percentage of this height with SEs (vertical bars). Numbers at the top of columns indicate the same as those in Fig. 4.

Fig. 7. Effects of sodium vanadate and ouabain on K-depolarized preparations of the guinea-pig mesenteric artery. Either drug was applied during the course of K-Tyrodé's solution (K-T)-induced contracture.

Fig. 8. Effects of DIDS on sodium vanadate- and ouabain-induced contractions of WKY aorta. 10^{-3} M DIDS was applied at a peak of 10^{-3} M sodium vanadate ($\text{NaVO}_3 10^{-3}$) or 10^{-3} M ouabain ($\text{Ouab } 10^{-3}$)-induced contraction.

ouabain did not show any contractile action but it showed no or weak relaxing action in aorae (data was not shown).

Next, effects of DIDS, an anion transport inhibitor, on vanadate- and ouabain-induced contractions were studied. In these experiments, DIDS was applied during the course of both contractions, since it was quite difficult to obtain the ouabain-induced contraction in the absence and presence of ouabain in the same preparation, and since DIDS is also known to block the contraction when applied during vanadate-induced contraction (Shimada *et al.*, 1986). As shown in Fig. 8, 10^{-3} M DIDS completely relaxed 10^{-3} M sodium vanadate-induced contraction of WKY aorta. Ouabain-induced contraction, on the other hand, was not blocked by DIDS of the same concentration, but DIDS induced a phasic contraction when applied in the presence of ouabain. Similar blocking effects of DIDS on sodium vanadate-induced contraction were observed in the guinea-pig aorta.

3. Effects of verapamil and Ca removal.

Fig. 9 shows the effects of verapamil on sodium vanadate- and ouabain-induced contraction of aortae. When 10^{-5} M verapamil was applied during the 10^{-3} M sodium vanadate-induced contraction, marked relaxing responses were observed in WKY and SHRSP aortae. In the guinea-pig aorta, on the other hand, no change in tension was brought about by the same concentration of verapamil.

Similar results were obtained in ouabain-induced contraction of the same preparation. The ouabain-induced contractions of WKY and SHRSP aortae were relaxed by verapamil, while those of the guinea-pig aortae were not significantly influenced by verapamil.

Removal of Ca from modified Tyrodé's solution abolished ouabain-induced contractions of aortae (Fig. 10). In another series of experiments, it was shown that no tension development occurred in aortae of any of the animal species examined in the absence of Ca, even after prolonged application of ouabain up to 90 minutes. The addition of Ca to Ca-free solution in

Fig. 9. Effects of verapamil on sodium vanadate- and ouabain-induced contractions of aortae of various animal species. 10^{-6} M verapamil was applied during the course of contractions by both agents. 10^{-3} M sodium vanadate (NaVO_3) was used in all aortae. Ouabain concentration differed in guinea-pig aortae and in WKY and SHRSP aortae, as stated in the figure (in M).

Fig. 10. Effects of Ca-removal on sodium vanadate- and ouabain-induced contractions in guinea-pig aorta. Ca with downward arrow and Ca-free indicate the removal of Ca from incubation medium. Ca with upward arrow indicates the application of 2 mM Ca in the presence of ouabain (10^{-4} M) or sodium vanadate (10^{-3} M). W, washing out with modified Tyrode's solution.

the presence of ouabain caused immediate development of tension to a level similar to that of ouabain-induced contraction in the presence of Ca. In the preparation shown in Fig. 10, the recovery after washing out of ouabain was particularly slow. The removal of Ca during the course of the recovery caused prompt relaxation of the preparation.

Ca removal also reduced vanadate-induced contraction of the aortae of all animal species tested, but it did not block the contractions completely. As shown in Fig. 10, the guinea-pig aorta responded to sodium vanadate in the absence of Ca, showing a contraction height of about one-third that of the control sodium vanadate-induced contraction observed in the presence of Ca. The addition of Ca to Ca-free Tyrode's solution in the presence of sodium vanadate induced immediate tension development to the level of the control sodium vanadate-induced contraction. Similar results were obtained in aortae of WKY and SHRSP, though the contrac-

tions in the absence of Ca were markedly smaller than those of the guinea-pig aorta; vis-à-vis the dependence on extracellular Ca were greater in WKY and SHRSP aorta.

Discussion

Both vanadate and ouabain, which are known as potent inhibitors of Na, K-ATPase, induce contraction of vascular smooth muscles, as described in the introduction. It was revealed in the present experiments that these agents showed contractile actions in various types of vascular smooth muscle in a similar fashion, that is, higher contractile action in aortae of guinea-pigs, WKY and SHRSP, and lower action in the mesenteric arteries of these animals. The time to peak tension, however, differed greatly in the vanadate-induced contractions and in the ouabain-induced contractions. This may indicate some difference in the mechanism (s) of the action.

It has been reported in a certain number of vascular tissues that ouabain induces the release of noradrenaline from intrinsic sympathetic nerves (Bonaccorsi *et al.*, 1977; Palatý, 1980; Katsuragi and Su, 1982; Aarhus *et al.*, 1983; Török *et al.*, 1984) and that the contractions by ouabain are mediated by released noradrenaline (Karaki and Urakawa, 1977; Karaki *et al.*, 1978; Toda, 1980; Katsuragi and Su, 1982; Aarhus *et al.*, 1983). However, the degree of involvement of released noradrenaline in ouabain-induced contraction varies among types of vascular tissues (Karaki *et al.*, 1978). In the present experiments, it was demonstrated that ouabain-induced contractions of the aortae of the guinea-pigs, WKY and SHRSP were depressed by the treatment with phentolamine. These results were inconsistent with those reported by Karaki *et al.* (1978), and indicated the involvement of released noradrenaline in the initiation of contraction by ouabain. Difference in the concentration of phentolamine (5×10^{-5} M in the present experiment and 10^{-6} M in the experiment reported by Karaki *et al.*, 1978) and of ouabain (10^{-4} M and 5×10^{-5}) may be the cause of the inconsistency.

The contractions of aortae of all animals by sodium vanadate, on the other hand, were not affected significantly by the treatment with phentolamine of the same concentration. This indicates that the vanadate-induced contractions of aortae are not mediated by intrinsic noradrenaline. These results are in agreement with those reported in the aortae of Dahl rats (Rapp, 1981) and also in the guinea-pig aorta (Ozaki and Urakawa, 1985). Thus, it has been clarified that the major part of ouabain-induced contraction and all of vanadate-induced contraction are brought about by the direct action of these agents on vascular smooth muscles.

Vanadate has been thought to act inside smooth muscle cells, since its action was depressed by anion transport inhibitors such as DIDS or 4 acetamino-4'-isothiocyano-2, 2'-disulfonic stilbene (SITS). (Rapp, 1981; Hudgins and Bond, 1981; Ueda and Urakawa, 1983; Nayler and Sparrow, 1983; see also Schwartz and Adams, 1980). It was also demonstrated in the present experiment that DIDS blocked completely vanadate-induced contraction of the aortae of the guinea-pigs, WKY and SHRSP, while ouabain- and K-induced contractions were not blocked. Thus, it can be concluded that the effect of DIDS was not nonspecific one and that vanadate should penetrate cell membrane for the exertion of its action. It has been known that ouabain binds sites located on the surface of cell membrane (see Akera and Brody, 1978; Schwartz and Adams, 1980; Wallick *et al.*, 1982; Anner, 1985).

The potentiation of vanadate-induced contraction by depolarization in the presence of elevated-K, therefore, may be explained by increased membrane permeability to vanadate. The increase in Ca permeability by depolarization and the inhibition of extrusion or sequestration of influxed Ca by vanadate (Wibo *et al.*, 1981; Popesuc and Ignat, 1983; Raeymaekers *et al.*, 1983) might also explain the potentiation by elevated K⁺. The elevation of K-concentration also potentiated ouabain-induced contraction but to a lesser extent and also in a different fashion from the potentiation of vanadate-induced contraction. Unnecessity of membrane permeation for the exertion of the action, interaction with elevated-K⁺ and inability of inhibiting Ca extrusion or sequestration might account for the lesser extent of the potentiation. However, further studies are required to explain these differences.

Ouabain-induced contractions of all aortae of all animal species were abolished by the removal of extracellular Ca. Similar observations have been reported in rabbit aorta (Briggs and Shibata, 1966), dog cerebral, basilar and mesenteric arteries (Toda, 1980) and guinea-pig aorta (Ozaki *et al.*, 1978). These may indicate that the inward movement of Ca is the first incidence of ouabain-induced contraction. It has also been reported in rabbit aorta that ouabain increased ⁴⁵Ca uptake (Briggs and Shibata, 1966). Since it has been observed in various vascular smooth muscles that ouabain causes depolarization of membrane (Hendrickx and Casteels, 1974; Casteels *et al.*, 1977; Kuriyama and Suzuki, 1978; Reiner, 1978; Belardinelli *et al.*, 1979; Mulvany *et al.*, 1984) depolarization dependent Ca influx may occur. However, the effects of Ca-antagonist do not fully support this assumption, as will be discussed below.

Vanadate-induced contractions, on the other hand, were relatively resistant to the removal of extracellular Ca, and contractions of the amplitudes of thirty to forty percent of the control were observed after soaking the preparations in Ca-free solution for longer than 30 minutes. Vanadate itself does not initiate but rather depresses the contraction of skinned vascular smooth muscle (Shimada *et al.*, 1986). The contraction by vanadate in the absence of extracellular Ca, therefore, might be initiated by released Ca from intracellular binding sites. The part of the contraction which disappeared in the absence of Ca, then, might be brought about by influxed Ca from extracellular space.

Effects of verapamil were similar in vanadate- and ouabain-induced contraction; that is, they were ineffective in the guinea-pig aorta and effective in WKY and SHRSP aortae. Ineffectiveness of verapamil or D600 has been observed in K-free-induced contraction (Ozaki and Urakawa, 1979) and ammonium vanadate-induced contraction (Ozaki and Urakawa, 1980) of the same smooth muscle, the guinea-pig aorta. Since verapamil has been known to block the depolarization-operated Ca channel (Fleckenstein, 1983), both the ouabain-induced and the extracellular Ca dependent part of vanadate-induced contraction of the guinea-pig aorta might be initiated by influxed Ca through pathways other than voltage-dependent channels. In rats, on the other hand, the major part of both ouabain- and vanadate-induced contraction might be brought about by influxed Ca through a potential operated Ca channels, though the depolarization is not expected to be as high as that induced by high-K (Hendrickx and Casteels, 1974; Casteels *et al.*, 1977; Kuriyama and Suzuki, 1978; Reiner, 1978; Mulvany *et al.*, 1984 for ouabain and Shimamura *et al.*, 1986 for vanadate).

Thus, ouabain-induced contractions of all vascular smooth muscles can be explained by the influx of Ca through both voltage dependent and independent Ca channels. The degree of contribution of both Ca channels varies among animal species. A similar explanation can be applied to vanadate-induced contractions. However, Ca released from intracellular binding sites is also involved in vanadate-induced contractions in addition to Ca influxed from extracellular space. The inhibition of Ca-ATPase and Ca-uptake of sarcoplasmic reticulum reported in gastric and intestinal smooth muscle (Wibo *et al.*, 1981; Raeymaekers *et al.*, 1983) may be a cause of the net release of Ca. Inhibition of Ca-ATPase and Ca-uptake of the sarcolemmal fraction by vanadate reported in smooth muscles (Wibo *et al.*, 1981; Popescu and Ignat, 1983; Raeymaekers *et al.*, 1983) can also be a cause of tension development inhibiting extrusion of influxed Ca.

At high concentrations, vanadate inhibits tension development of skinned preparation of hog carotid artery (Peterson, 1980) and rabbit mesenteric artery (Shimada *et al.*, 1986). This action may be the cause of the relaxation of depolarized preparation by vanadate. Membrane permeability to vanadate might increase under the depolarized state in K-Tyrode's solution, and consequently, intracellular vanadate concentration would be increased to the level which inhibits the contraction induced by high-K. Ouabain does not possess such inhibiting action on contractile protein, and therefore shows no relaxing but rather contractile action increasing Ca influx further.

Thus, although similar potency of the action of ouabain and vanadate was observed among various types of vascular smooth muscles, it can be concluded that the mechanisms of the contractile action differ in ouabain- and in vanadate-induced contractions. Ouabain induces contraction increasing Ca influx, which is mediated by the inhibition of Na, K-ATPase. Vanadate, on the other hand, induces contraction both by increasing Ca influx and releasing intracellular bound Ca. The inhibition of Ca-ATPase of membrane and sarcoplasmic reticulum may be involved in the action of vanadate.

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