DUAL ACTIONS OF VANADATE ON HIGH K-INDUCED CONTRACTION IN GUINEA-PIG TAENIA COLI

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Abstract—Effects of vanadate (NH₄VO₃: VAN) on tension development, membrane potential and cellular Na content were investigated in guinea-pig taenia coli depolarized by 62.7 mM KCl solution. VAN (10⁻⁴–10⁻³ M) caused a transient increase in the K developed tension followed by a relaxation. The VAN-induced contraction was observed even in a low Ca (0.13 mM) solution but was inhibited by the removal of external Ca. After the addition of verapamil (5×10⁻⁸, 10⁻⁷ M), VAN still produced a contraction. Further, the VAN-induced contraction was observed in 142.2 mM KCl (Na 11.9 mM) solution containing ouabain (10⁻⁴ M). On the other hand, the effect of VAN to relax the K-induced contraction was dependent on the concentration of VAN. In low Na (choline-substituted) solution, the VAN-induced relaxation was decreased. VAN increased cellular Na content of the depolarized muscle, and a correlation was obtained between the cellular Na accumulation and the relaxation. These results suggest that the relaxation is mainly attributable to the accumulation of Na following the inhibition of the Na pump, while the contraction is independent of the inhibition of the Na pump and less sensitive to the external Ca than the K-induced process.

Ouabain, a cardioactive glycoside, has been known to relax the sustained contraction induced by a high concentration of KCl in intestinal smooth muscle (1–6). Bose (6) suggested that the inhibitory effect of ouabain is attributable to the accumulation of Na following the inhibition of the Na pump. Kishimoto et al. (7) showed a quantitative relationship between cellular Na accumulation and relaxation caused by ouabain in the depolarized smooth muscle of guinea-pig taenia coli and confirmed the above concept.

Recently, biochemical actions of vanadate (VAN), vanadium in the +5 oxidation state, were extensively studied (8). Cantley et al. showed that VAN is a potent inhibitor of the Na,K ATPase isolated from striated muscle (9) and suggested that, unlike cardioactive glycosides, VAN inhibits the red cell Na,K ATPase from the cytoplasmic side (10).

In the present study, we investigated the effects of VAN on tension development, membrane potential and cellular Na content in the depolarized smooth muscle of guinea-pig taenia coli and compared the effects with those of ouabain.

MATERIALS AND METHODS

Preparations: Male guinea-pigs weighing
300–400 g were stunned and bled, and strips of taenia coli approximately 15 mm in situ length were isolated. Each strip was then suspended in an organ bath containing 20 ml of normal physiological solution and equilibrated for 30 min. In some experiments, muscle strips stored overnight at 5 °C were used; the cold stored preparations were equilibrated at 37°C for at least 90 min before the experiment.

**Solutions:** Table 1 shows the composition of the solutions used in the present experiments. All the solutions were aerated with a 95% O₂–5% CO₂ gas mixture at 37°C and pH 7.2 unless otherwise stated.

**Mechanical and electrical measurements:** Muscle tension was recorded under an isometric condition with a strain-gauge transducer (Nihon Kohden). Each strip was maintained under a stable tension of approximately 0.5 g. The magnitude of the tonic contraction induced by 62.7 mM KCl (62.7 K) solution was used as a reference response (100%).

Membrane potential was recorded by a single sucrose gap method similar to that described by Bulbring and Burnstock (11). All the experimental conditions were introduced after the development of spontaneous electrical activities. The electrophysiological study was carried out at 32°C since the spontaneous activity was constant at this temperature.

**Determination of Na content:** Cellular Na content was measured by the modified "lithium method" originally developed by Friedman (12) for rat tail artery and later applied to taenia coli (7). Strips of taenia coli were fixed to glass holders under a stable tension of approximately 0.5 g and stored overnight at 5°C. They were then equilibrated at 37°C for at least 90 min before starting the experiment. After a desired incubation period in a test solution, the strips were washed with Li solution at 0°C for 60 min to remove extracellular Na. Then, the strips were blotted on a filter paper, weighed, digested with 0.5 ml of a HClO₄ and HNO₃ (1:1) mixture and evaporated to dryness overnight at 180°C. The dried samples were dissolved in a diluting fluid containing 0.01 N HCl and 1 g/l CsCl. The Na concentration in the samples was determined with a flame photometer (Hitachi).

**Drugs:** NH₄VO₃ (Wakoh Junyaku), verapamil (Eisai), ouabain (Merck) and atropine (Tokyo Kasei) were used. Stock solutions of verapamil and ouabain were made and an appropriate amount of the stock solution was added to the test solution.

Differences were tested for significance with the Student's t-test. Results are presented as the mean value±S.E.M. for n experiments.

**RESULTS**

In the guinea-pig taenia coli, a solution containing 62.7 mM KCl produced a sustained contraction of about 8 g. VAN (1×10⁻⁴–1×10⁻³ M), applied to the muscle during the
K-induced contraction, transiently enhanced the K-induced contractile tension after a latency of 0.5 to 3 min and was subsequently relaxed (Fig. 1-a). The effect of VAN to relax the K-induced contraction was dependent on the concentration of VAN (Fig. 1-b). The high K solution induced a depolarization of the membrane by about 15 mV, and this level was maintained. VAN (1 × 10^{-3} M), added during the depolarization, had little effect on the membrane potential (Fig. 2).

The contractile effect of VAN: First, the effects of changing the external Ca concentration and organic Ca antagonist on the contractile effect of VAN were investigated. Ca depletion abolished the contractile effect of VAN as well as the K-induced contraction. In a low Ca (0.13 mM) solution, however, VAN (1 × 10^{-3} M) produced a large contractile tension while the K-induced contraction was small (Fig. 3-a). In the presence of verapamil, VAN still produced contractions; and the magnitudes were 18.3% (n=5) and 31.7% (n=4) at the verapamil concentrations of 10^{-7} M and 5 × 10^{-8} M, respectively, while those for K-contraction were 7.1% (n=5) and 17.7% (n=4), respectively. The above results suggest that the VAN-induced contraction is less sensitive to the external Ca than the K-induced one.

The effects of external K concentration on the VAN-induced contractile tension were investigated under a low Ca (0.13 mM) condition. Under such a condition, the K-induced contraction was almost negligible, and as a result, the VAN-induced contraction was clearly demonstrated. VAN (1 × 10^{-3}) produced a large contractile tension in the physiological K (5.4 mM) solution and even in the 142.2 mM K (Na, 11.9 mM) solution (Fig. 3-a). These results are summarized in Fig. 3-b. These results suggest
that VAN-induced contractile tension is not affected by a change in the external K concentration.

It has been reported that $1 \times 10^{-4}$ M ouabain produced a maximum inhibition of the Na pump in guinea-pig taenia coli (7, 13). In the present study, VAN (1 $\times 10^{-3}$ M) was applied in the 62.7 mM KCl solution containing $1 \times 10^{-4}$ M ouabain under the low Ca condition (Ca, 0.13 mM). Ouabain inhibited the K-induced contraction, but VAN still produced a contraction in the presence of ouabain (Fig. 4-a). Further, ouabain was applied to the Na deficient-142.2 mM KCl (Na 11.9 mM)-induced contraction under the low Ca condition. The result shows that ouabain had little effect on the K-induced contraction, but the subsequent application of VAN (1 $\times 10^{-3}$ M) still induced a contractile tension (Fig. 4-b). In a low Na (11.9 mM Na and 106.8 mM choline) solution with 62.7 mM KCl and 2.5 mM Ca, the muscle showed a phasic contraction followed by a sustained one. Ouabain (2 $\times 10^{-5}$ M) had little effect on the sustained contraction, although VAN (1 $\times 10^{-3}$ M) induced a transient increase in the muscle tension (see Fig. 9). These data suggest that the contractile effect of VAN is independent of the inhibition of the Na pump.

The relaxation by VAN: The effect of VAN on Ca-induced contraction in taenia coli depolarized by the 62.7 mM KCl solution was investigated. After a 2 hr incubation of taenia coli in 62.7 mM KCl (2.5 mM, Ca)
solution with or without $5 \times 10^{-4}$ M VAN, external Ca concentration was decreased to 0.13 mM, resulting in a decrease in tension. Subsequently, cumulative addition of Ca up to 9 mM produced a graded contraction (Ca was applied after each contraction had reached maximum as shown in Fig. 5-a). The concentration-response curve for Ca shifted to the right in the presence of VAN (Fig. 5-b), suggesting that pretreatment by VAN inhibits the Ca-induced contraction.

Figure 6 shows the effects of VAN and ouabain on the cellular Na content estimated by the "lithium method" (12). The cellular Na content of taenia coli in physiological and high K (62.7 mM) solutions were $5.4 \pm 0.5$ mmol/kg wet wt. ($n=6$) and $6.0 \pm 0.3$ mmol/kg wet wt. ($n=6$), respectively. In the physiological medium, the Na content increased to $44.2 \pm 3.2$ mmol/kg wet wt. ($n=6$) at 1 hr and to $69.3 \pm 1.3$ mmol/kg wet wt. ($n=6$) at 3 hr after the addition of ouabain ($1 \times 10^{-6}$ M). In the high K medium, ouabain also showed a similar effect. VAN ($1 \times 10^{-3}$ M) added to the physiological solution changed the Na content slightly ($7.6 \pm 0.4$ mmol/kg wet wt., $n=6$ at 30 min and $8.6 \pm 0.4$ mmol/kg wet wt., $n=6$ at 1 hr).

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**Fig. 5.** Effects of various concentrations of Ca on the muscle tension depolarized by 62.7 mM KCl (Ca 2.5 mM) in the absence (a) or presence (b) of VAN ($5 \times 10^{-4}$ M). Ca concentration was lowered to 0.13 mM at the first dot and then Ca was added cumulatively up to 9 mM. (c) Symbols represent the mean values±S.E.M. of $b/a \times 100$ for 3–5 experiments in (a) and (b). Ordinate: Relative tension (%) to 62.7 mM Ca-induced contractile tension in the presence of 2.5 mM Ca. Abscissa: External Ca concentration (mM).

**Fig. 6.** Effects of ouabain and VAN on cellular Na content of taenia coli in 5.4 mM KCl or 62.7 mM KCl solution. Symbols represent the results with ouabain (▲) and VAN (●) in 5.4 mM KCl solution and ouabain (○) and VAN (●) in 62.7 mM KCl solution. The Na content was determined by the "lithium method". Abscissa: Time (hr). Ordinate: Cellular Na content (mmol/kg wet wt.). Given are the mean values±S.E.M. for 6 experiments. (▲): Control in 5.4 mM KCl solution. (●): Control in 62.7 mM KCl solution. VAN: $10^{-3}$ M. OUA: $10^{-5}$ M.
mmol/kg wet wt., n=6 at 3 hr). However, VAN (1×10⁻³ M) added to the 62.7 mM KCl solution significantly (P < 0.01) increased the cellular Na content to 37.5±3.7 mmol/kg wet wt. (n=6) after a 2 hr incubation. These data suggest that VAN inhibits the Na pump only when external K is high.

Figure 7 summarizes the time courses of the changes in muscle tension and Na content induced by VAN in the 62.7 mM KCl solution. Cellular Na content increased on the application of VAN and reached a plateau after 2 hr. It seems likely that the muscle relaxation induced by VAN correlates with the accumulation of cellular Na, but the additive contraction does not. Therefore, the relationship between muscle tension and cellular Na content was examined. Muscle strips were treated for 2 hr with the 62.7 mM KCl solution containing various concentrations of VAN. At the end of the treatment, muscle tension and cellular Na content were measured. A good correlation was obtained between the relaxation and the cellular Na content as shown in Fig. 8. The cellular Na content for 50% relaxation of the K-contraction was 16.7 mmol/kg wet wt.

In the low Na (11.9 mM Na and 106.8 mM choline) solution with 62.7 mM KCl, VAN (1×10⁻³ M) induced a very slow relaxation...
reaching about 60% of the original tension in 4 hr, while ouabain had little effect (Fig. 9). Similar results were obtained in the K-substituted (K, 142.2 mM) solution.

**DISCUSSION**

**The contractile effect by VAN:** The present results showed that VAN, an inhibitor of the Na, K ATPase (9), has dual actions in depolarized taenia coli: the additive contraction and the subsequent relaxation.

In the 142.2 mM KCl solution, the VAN-induced contraction was somewhat larger than that in the lower concentration of K. Further, the VAN-induced changes in muscle tension were not accompanied by a change in membrane potential. Therefore, it is suggested that the VAN-induced contractile effect is independent of the further depolarization of the membrane.

Furthermore, the contractile effect by VAN was examined in the relation to the Na pump activity. The results showed that VAN produced a contraction even in the presence of $1 \times 10^{-4}$ M ouabain, which maximally inhibits Na, K ATPase activity (13). VAN induced contractile effects in Na deficient solutions (substituted by KCl or choline), while ouabain did not. These results suggest that the contractile effect of VAN is not due to an inhibition of the Na pump.

As demonstrated in Fig. 2 the contraction induced by VAN was less sensitive to either the decrease in external Ca or the application of an organic Ca antagonist. Further, VAN was found to develop tension in completely depolarized smooth muscle (142.2 mM, K). In intestinal smooth muscles, the contractile response is suggested to be dependent on the utilization of either external Ca, as in high K-induced contraction, or cellular bound Ca, as in the carbachol-induced contraction (14–16). The former contraction is rapidly abolished by the removal of external Ca or application of Ca antagonists. The present results suggest that the Ca-dependency of the VAN-induced contraction is different from that of the K-induced one. Ozaki and Urakawa (17) reported that the characteristics of the contraction induced by VAN and norepinephrine were very similar in vascular smooth muscle. It is known that the norepinephrine-induced response in vascular smooth muscle is due to a release of Ca from the cellular storage site (18). VAN is also reported to be an inhibitor of the Ca ATPase in various tissues (19–21). Therefore, both these possibilities may also exist in taenia coli.

However, further experiments are needed to clarify the mechanism of the VAN-induced contraction.

**The relaxation by VAN:** The VAN-induced relaxation was decreased in low Na (11.9 mM Na) solution, and a correlation was found between the VAN-induced relaxation and accumulation of cellular Na.

Kishimoto et al. (7) reported a similar correlation between ouabain-induced relaxation and accumulation of cellular Na in depolarized taenia coli and suggested that the increased cellular Na inhibits the K-induced Ca influx. Therefore the VAN-induced relaxation is also probably attributable to the increased cellular Na. Ouabain-induced relaxation was not seen in low Na solution, (6, 7) but VAN still induced a slow and small relaxation in the low Na solution. Therefore, it seems likely that a part of the VAN-induced relaxation may be due to another inhibitory mechanism, unlike ouabain.

Cantley et al. (10) reported that VAN enters the red cell under a physiological K concentration and suggested that the site for inhibition by VAN of the Na, K ATPase was located at the internal surface of the cell membrane. In the present study, however, VAN scarcely affected cellular Na contents in physiological K solution. On the other hand, VAN increased significantly the cellular
Na content in a high K solution. Beaugé et al. (22) have also reported that an inhibitory effect of VAN on K influx was observed in human red cells only if external K concentration was significantly higher (above 30 mM) than physiological concentrations. In taenia coli, it is also suggested that high external K is needed for the VAN-induced inhibitory effect on the Na pump.

In summary, VAN has dual actions on the K-depolarized smooth muscle of taenia coli. VAN exhibited a transient increase in the K developed tension followed by a relaxation. These results suggest that the relaxation is mainly attributable to the accumulation of Na following the inhibition of the Na pump, while the contraction is independent of the inhibition of the Na pump.

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