EFFECTS OF OUABAIN ON HIGH-K INDUCED CONTRACTIONS
OF VARIOUS SMOOTH MUSCLE TISSUES IN THE GUINEA-PIG

Tadashi KISHIMOTO and Norimoto URAKAWA
Department of Veterinary Pharmacology, Faculty of Agriculture,
University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Accepted February 17, 1982

Abstract—The effects of ouabain on contraction, cellular Na concentration, and $^{45}$Ca uptake of various smooth muscle tissues depolarized by high-K (62.7 mM) solution were studied. In the taenia coli and ileum isolated from guinea-pig, ouabain ($2 \times 10^{-6}$ M – $5 \times 10^{-5}$ M) increased cellular Na concentration and strongly inhibited K-contraction. A good correlation between cellular Na concentration and inhibition of tension was obtained both in the taenia coli and ileum. In the portal vein, aorta, and uterus, ouabain failed to induce relaxation and slightly increased the cellular Na. A long-time exposure to ouabain ($1 \times 10^{-3}$ M) attenuated the response to high-K in the portal vein and uterus but not in the aorta. The inhibitory effect of ouabain in the taenia coli and ileum was antagonized by an increase in external Ca, and the inhibition was accompanied by a decrease in cellular $^{45}$Ca content. A possibility exists that excess cellular Na inhibits K-induced Ca influx, although there is a tissue-specific difference. The data also suggest that the inhibitory effect of Na-accumulation is dissimilar to that of verapamil with respect to the tissue-specific difference.

Na-Ca interactions in smooth muscle involve a variety of cellular mechanisms that cannot be adequately described by the single hypothesis of a Na-Ca exchange carrier (1). In the preceding paper (2), we reported that ouabain-induced relaxation of the guinea-pig taenia coli in high-K solution was closely correlated with the increase in cellular Na. The observation suggests that the increased intracellular Na in some way inhibits the high-K induced inward movement of Ca. In other smooth muscle preparations such as guinea-pig ileum (3), guinea-pig and rat portal veins (4–6), and rabbit pulmonary artery (7), mechanical responses to high-K solution are also highly dependent upon the extracellular Ca concentration and are very sensitive to the Ca antagonist, verapamil. Therefore, it seems worthwhile to investigate whether in different smooth muscle tissues the effect of ouabain is similar to the result with taenia coli.

In the present study, we have investigated the effects of ouabain on K-induced contractions in guinea-pig taenia coli, ileum, portal vein, aorta and uterus; and the results are compared with each other. Experiments were performed to study alterations in tension development, extracellular space, dry weight/wet weight, cellular Na concentration, and $^{45}$Ca uptake following the treatment with ouabain. Some important and interesting differences were found in various types of smooth muscle preparations.
MATERIALS AND METHODS

Preparations: Guinea-pigs of either sex weighing between 300–400 g were bled after stunning; and the taenia coli, ileum, portal vein, and aorta were removed. Female guinea-pigs were given an injection of estradiol (100 µg/kg, s.c.), and after 20 hr, the strips of the uterus were removed. Longitudinal preparations of the ileum and uterus were removed from the circular muscle underlying them by a method similar to that described by Rang (8). Strips of the portal vein and aorta were cut longitudinally and helically, respectively. For the determination of cellular Na and Ca contents and extracellular space, tissues were cut longitudinally, and rectangular strips weighing 3–10 mg were prepared. Some strips were stored overnight in a refrigerator (3°C). The strips were incubated in a normal physiological salt solution at 37°C for more than 1 hr after the cold storage. The muscle contractility was not altered as compared with that of fresh strips.

Solutions: The normal physiological salt solution (PSS) was of the following composition (mM): NaCl, 136.8; KCl, 5.4; CaCl₂, 2.5; MgCl₂, 1.0; NaHCO₃, 11.9; and glucose, 5.5. In order to minimize both the decrease in external Na concentration and increase in osmolality, high-K (62.7 mM) solution was made by increasing KCl concentration to 62.7 mM and reducing NaCl concentration to 106.8 mM. Bathing solutions were kept at 37°C and equilibrated with 95% O₂ and 5% CO₂ at pH 7.2. Ca-free solution was made by omitting CaCl₂ from the original medium without any substitution for osmolality.

Tension Recordings: The contractile tension of muscle strips was recorded isometrically. The strip of taenia coli (about 15 mm long, 2 mm wide), the longitudinal strip of the ileum (about 15 mm long, 10 mm wide), the longitudinal strip of the portal vein (about 7 mm long, 4 mm wide), the helical strip of the aorta (about 12 mm long, 4 mm wide), or the longitudinal strip of the uterus (about 15 mm long, 6 mm wide) was suspended in an isolated organ bath (20 ml) and attached to the arm of a strain-gauge transducer (Nihon Kohden). The loading tension applied to the strips of the taenia coli, ileum, portal vein, and uterus was about 0.5 g and that for the aortic strip was 1 g. The muscles were allowed to equilibrate in normal PSS for at least 1 hr until the response to high-K solution became stable. The magnitude of the tonic contraction in response to the high-K (62.7 mM) solution was considered as a reference response (100%). In the cases with the portal vein and aorta, some experiments of tension were conducted in the presence of phentolamine (2x10⁻⁶ M) in order to eliminate any contraction due to neuronal release of noradrenaline.

Determination of cellular Na concentration: Cellular Na content in the muscle cells was determined by the "Li-method" originally described by Friedman (9), and the experimental apparatus and procedure were the same as those described by Kishimoto et al. (2). During the washout of all the preparations with cold Li-solution (LiCl, 169.5; CaCl₂, 2.5; MgCl₂, 1.0; LiHCO₃, 11.9 and glucose, 5.5 mM), a part of the Na from the tissues was rapidly lost, and this was followed by a rapid Li uptake while the remaining Na was lost very slowly after 30 min as a single exponential process. Therefore, we estimated that the residual Na content after a 30 min-washout with Li-solution at 0.5°C represents the cellular Na level. After the 30 min-washout of the tissues, they were blotted between filter papers, weighed, and ashed in a quartz tube with 0.5 ml of a mixture (1:1) of HNO₃ (61%) and HClO₄ (60%), overnight at 180°C. The dried samples were then redissolved in 0.01 N HCl solution. Na
content in the samples was measured by flame photometry. Cellular Na concentration was calculated by the following equation:

\[
\text{Cellular Na (mmol/l cell water) = } \frac{\text{Li-inaccessible Na (mmol/kg wet wt.)}}{1 - \text{ECS-Dry weight/Wet weight}}
\]

The dry weight was obtained by drying the tissue for 20 hr at 95°C. Tension recordings and determinations of Na content were made on separate strips, except where specifically indicated.

**Determination of extracellular space:** The size of the extracellular space (ECS) was determined by the uptake of an extracellular space marker, \(^{14}\text{C}-\text{sorbitol} (2 \mu\text{Ci/ml, The Radiochemical Centre, England}). The muscles were incubated in the high-K solution with or without \(1 \times 10^{-5} \text{ M ouabain}\) for 60 min. Muscles were exposed to the radioactive solution for the last 20 min of the incubation. The muscles were then blotted, weighed, and placed in scintillation vials containing 0.5 ml of tissue solubilizer (Lumasolve Lumac Systems A.G.) and digested overnight at 55°C. The solubilized sample was mixed with 5 ml of scintillator (Lumagel, Lumac Systems A.G.), and the radioactivity was determined using a liquid scintillation spectrometer (Tri-Carb 3380, Packard Co.). The size of the ECS was expressed as a percentage of the tissue volume.

\(^{45}\text{Ca Uptake:}\) \(^{45}\text{Ca}\) uptake into the muscle cells was measured by a modified “La-method” which was developed by Karaki and Weiss (10). After incubation with various test solutions containing a tracer amount of \(^{45}\text{CaCl}_2 (5 \mu\text{Ci/ml, New England Nuclear})\), the strips were washed in a La-solution (73.8 mM \text{LaCl}_3, 5.5 mM glucose, and 11.9 mM Tris-HCl, pH 7.2) at 0.5°C for 60 min to remove the extracellularly bound \(^{45}\text{Ca}\). The radioactivity of the sample was determined by the same method as that for \(^{14}\text{C}-\text{sorbitol}\). Cellular \(^{45}\text{Ca}\) content was expressed as mmol/kg wet wt.

**Drugs:** The drugs used were ouabain (Merck), verapamil (Eisai), atropine sulphate, propranolol hydrochloride (Tokyo Kasei), phentolamine methylate (Ciba Geigy), and \(\beta\)-estradiol (Sigma).

**RESULTS**

**A. Effects of ouabain and verapamil on K-contractions:** Exposure of the taenia coli, ileum, portal vein, and uterus to high-K

---

**Fig. 1.** Effects of ouabain \((1 \times 10^{-5} \text{ M})\) on K-contractions in the taenia coli, ileum, portal vein, aorta, and uterus.
(62.7 mM) solution resulted in a rapid phasic contraction (within 1 min) followed by a tonic contraction (Fig. 1). The magnitudes of the tonic contractions were about 8, 4, 0.7, and 1 g for the taenia coli, ileum, portal vein, and uterus, respectively. With the aorta, the excess K produced a gradual contraction which reached its maximum (about 1 g) after 20 min (Fig. 1). When high-K solution was exchanged with normal PSS or with a Ca-free high-K solution, the developed tension declined to the original level. The tonic contraction induced by 62.7 mM K was selected for study of relaxation in all the muscles since the contractile mechanism is mainly due to depolarization-induced Ca influx as described in a following paper (11).

Thirty minutes after exposure to high-K solution, ouabain (1 x 10^{-6} M) was applied to the depolarized smooth muscles of the taenia coli, ileum, portal vein, aorta, and uterus. These results are shown in Fig. 1. In the taenia coli and ileum, the muscle tension decreased gradually, reaching a new steady level (21.8±1.3%, 29.9±2.3% of the K-contraction, respectively) at 50 min. As shown in Fig. 2, the inhibitory effects were concentration-dependent (2 x 10^{-6} M - 5 x 10^{-5} M). In the portal vein, the muscle tension was transiently potentiated and this was followed by a gradual decline reaching a steady level similar to the K-contraction within 2 hr (Fig. 1). The potentiating effect was not inhibited by phentolamine (2 x 10^{-6} M).
M), but disappeared when all the external Na was replaced by sucrose or choline (plus 2×10^{-6} M atropine). In the aorta and uterus, ouabain failed to affect the high-K developed tension (Fig. 1). Although the results are not shown in the figure, the contraction developed by hyperosmotically adding 30 mM KCl was decreased by ouabain (1×10^{-5} M) in the uterus but not in the portal vein or aorta.

Thirty minutes after the application of high-K solution, verapamil was added cumulatively (1×10^{-8} M - 1×10^{-6} M). As shown in Fig. 3, verapamil produced a concentration-dependent relaxation of the K-contraction in various smooth muscle preparations. The concentrations of verapamil which inhibited the contraction by 50% in the taenia coli, ileum, portal vein, aorta, and uterus were 5.0×10^{-8} M, 4.4×10^{-9} M, 3.3×10^{-8} M, 2.9×10^{-7} M, and 1.6×10^{-8} M, respectively.

B. The effects of ouabain and verapamil on Ca-induced contractions: The inhibitory effects of ouabain on Ca-induced contractions in high-K solution were investigated.

**Fig. 4.** Effects of ouabain and verapamil on the contraction evoked by adding Ca cumulatively to the various tissues incubated in Ca-free high-K solution for 2 hr. Relative tension is expressed as percent of the K-contraction (Ca, 2.5 mM) which is plotted on the ordinate as a function of external Ca concentration on the abscissa. The tissues were pretreated with ouabain (■, 1×10^{-6} M) for 90 min, or with verapamil (●, 5×10^{-9} M) for 20 min. The control points are represented by open circles. Given are the mean values±S.E.M., as vertical bars of 4-12 experiments.
In Fig. 4, the results with ouabain are compared with those of verapamil, an organic Ca antagonist. After a 2 hr-incubation with a Ca-free solution containing 62.7 mM KCl, readmission of Ca was performed. Cumulative addition of Ca (0.25–10.0 mM) produced graded contractions in all the muscles, suggesting that the K-contractions are strictly external Ca-dependent. On the basis of the ED50 obtained from the figure, the dependence of muscle tension on external Ca seems to be higher in the uterus or portal vein than in the ileum, taenia coli, or aorta. Ouabain (1×10^{-5} M) was applied 90 min before and during the application of Ca, while verapamil (5×10^{-8} M) was applied 20 min before. In the taenia coli and ileum, the pretreatment with ouabain shifted the Ca concentration-response curve towards the right. Also, verapamil showed similar results in all the muscles. Therefore, the inhibitory effects of ouabain in the taenia coli and ileum are antagonized by external Ca, probably indicating that they interact at a site directly involved with Ca binding and (or) translocation. This site is presumably the Ca channel which is activated by high-K induced depolarization.

C. Relationship between cellular Na and tension: Possible changes in the size of the extracellular space (ECS) were investigated. As shown in Table 1, the sizes of the ECS in all the muscle preparations were almost unaffected by 1×10^{-5} M ouabain in high-K solution, except that there was a significant but small increase in the ECS of the taenia coli. In high-K solution, the dry weight/wet weight ratios in the taenia coli, ileum, portal vein, aorta, and uterus were 21.9±0.2, 21.4±0.2, 25.7±0.7, 25.0±1.3, and 20.0±1.3% (n=5), respectively.

Ouabain (1×10^{-5} M) was applied to the depolarized muscles after a 30 min-incubation in high-K solution, and then the changes in cellular Na concentration were determined with time. In high-K solution, the cellular Na concentrations of the taenia coli, ileum, portal vein, aorta and uterus were 17.6±0.7, 20.3±2.3, 30.4±3.1, 17.6±3.2, and 21.9±2.8 mmol/l cell water (n=6), respectively. As shown in Fig. 5A, ouabain induced more rapid and greater increases in cellular Na concentrations in the taenia coli and ileum than in the portal vein, aorta, and uterus. After 250 min, the maximum levels of Na accumulation reached in the taenia coli, ileum, portal vein, aorta and uterus were 93.5±3.3, 124.6±3.7, 92.0±4.7, 97.9±3.0, and 86.6±3.9 mmol/l cell water (n=4).

### Table 1. Effect of ouabain on the size of the ECS during K-contraction in the taenia coli, ileum, portal vein, aorta and uterus.

| Extracellular space (14C-sorbitol, %) | Normal PSS (4) | High-K solution (8) | High-K+ouabain (4) |
|-------------------------------------|---------------|-------------------|------------------|
| Taenia coli                         | 31.2±1.5      | 33.7±1.9          | 41.2±1.1*        |
| Ileum                               | 34.5±1.8      | 35.3±2.2          | 42.2±1.0         |
| Portal vein                         | 48.4±2.9      | 55.5±1.8          | 54.8±2.6         |
| Aorta                               | 57.7±1.5      | 56.2±1.4          | 54.4±1.8         |
| Uterus                              | 54.2±2.2      | 51.7±2.7          | 50.2±1.0         |

*: Significantly different from the ECS in high-K solution (P<0.05).
respectively. Figure 5B demonstrates the relationship between the increase in cellular Na and tension during exposure to ouabain for 50 min in high-K solution. In the taenia coli and ileum, a good correlation was obtained between cellular Na concentration and inhibition of the developed tension. In the taenia coli and ileum, the cellular Na concentrations required to inhibit K-contractions by 50% were approximately 62.5 and 60.0 mmol/l cell water, respectively. However, no correlation was seen in the other preparations.

In the next experiment, the effects of maximally accumulated cellular Na on K-contractions were investigated in the portal vein, aorta, and uterus. After an initial response to high-K solution, the strips were incubated in normal PSS containing 1×10^{-3} M ouabain for 4 hr in order to maximally load the strips with Na. Subsequently, they were again exposed to high-K solution in the presence of ouabain for 20 min. The comparison of the maximum responses to high-K solution before and after the ouabain-treatment is summarized in Table 2. In the portal vein and uterus, the development of

![Figure 5](image_url)

**Fig. 5.** A: Changes in cellular Na concentration induced by ouabain in K-depolarized preparations of taenia coli (■), ileum (○), portal vein (▲), aorta (△), and uterus (■). Ordinate: cellular Na concentration (mmol/l cell water). Abscissa: time (min) of exposure to 1×10^{-5} M ouabain. B: Relationship between cellular Na concentration and tension. Ordinate: relative tension (%). Abscissa: cellular Na concentration (mmol/l cell water). The symbols are the same as for A. Given are the mean values ±S.E.M. for 4-8 determinations.

| Table 2. Effects of pretreatment with higher concentration of ouabain on development of K-contraction and cellular Na concentration in the portal vein, aorta, and uterus. After a 4 hr-incubation in normal PSS containing 1×10^{-3} M ouabain, the muscle was exposed to high-K (82.7 mM). Immediately after the tension recording, the strips were removed from the apparatus and prepared for cellular Na determination. Experiments were performed in the presence of phentolamine (2×10^{-6} M). Numbers in the parentheses indicate the number of experiments. Given are the means±S.E.M. The data of (a) are from Fig. 5A. |
|---------------------------------|--------|--------|--------|
| **Control** | **Portal vein** | **Aorta** | **Uterus** |
| maximal response (g) to high-K solution | 0.59±0.06 | 1.11±0.10 | 2.64±0.32 |
| cellular Na\(^3\) (mmol/l cell water) | 30.4±3.1 | 17.6±3.2 | 21.9±2.8 |
| **Ouabain-treatment** | **Portal vein** | **Aorta** | **Uterus** |
| maximal response (g) to high-K solution | 0.27±0.02 | 1.18±0.06 | 1.01±0.16 |
| cellular Na (mmol/l cell water) | 123.4±3.7 | 145.2±19.8 | 128.4±13.4 |
K-contraction was attenuated by the ouabain-treatment to about 45% and 40% of the control, respectively, although these muscles had produced a small rise in the basal tone before the second exposure to high K. In the aorta, ouabain itself produced a sustained contraction which was as large as the K-contraction, and then the second exposure to high K further enhanced the ouabain-induced contraction by about 5%.

D. Changes in cellular $^{45}$Ca content: The effect of ouabain on the K-induced increase in cellular Ca content was investigated. These results are shown in Fig. 6. Muscle strips were incubated in high-K solution with or without $1 \times 10^{-5}$ M ouabain for 60 min, and then they were transferred to the radioactive solution. After loading with $^{45}$Ca for 30 min, the cellular $^{45}$Ca content was measured by a modified "La-method". In high-K solution, $^{45}$Ca uptake of the taenia coil, ileum, and aorta increased significantly compared with those in normal solution. In the case of the uterus, we could not obtain a significant increase in $^{45}$Ca content by the high-K solution because of the large variation. The K-induced $^{45}$Ca uptake was attenuated by ouabain in the taenia coli and ileum but not in the aorta or uterus. These results seem to be in good agreement with those of the tension experiment. Results with the portal vein could not be shown because of the scattering of the data obtained.

**DISCUSSION**

It is known that there are species differences in the sensitivities of the ileum (12), blood vessels (13), and uterus (14) to ouabain. Therefore, some difficulties are encountered in investigating the effects of ouabain on agonist-induced contractions by using various smooth muscle tissues isolated from different animal species (14–18). Previous studies have shown that the tonic contraction produced by a high-K solution is inhibited by ouabain in the guinea-pig taenia coli (2, 19–24) and ileum (25). In the present paper, we have found that the effects of ouabain on K-contraction are different in several smooth muscle tissues isolated only from the guinea-pig. Ouabain, accompanied with the accumulation of cellular Na, strongly inhibited the already established K-contractions in the taenia coli and ileum, but not in the portal vein, aorta, and uterus. This result may be partially related to a phenomenon that ouabain induced more rapid and greater increase in cellular Na concentrations in the taenia coli and ileum than in the portal vein, aorta, and uterus. A long-time exposure to a higher concentration ($1 \times 10^{-3}$ M) of ouabain (i.e., maximal accumulation of Na) attenuated the response to high K in the portal vein and uterus, but not in the aorta.

Our recent study (2) suggests that ouabain which inhibits the tonic contraction of the taenia coli depolarized by high-K has the following properties: 1) no effect on K-
induced membrane depolarization, 2) the inhibitory effect depends on the concentration of ouabain, 3) the dependence on the length of period of the application of ouabain, 4) the correlation between the accumulation of cellular Na and the inhibition, 5) the lack of ouabain-inhibition in Na-free, high-K solution, 6) the antagonization by raising external Ca and 7) the inhibition of $^{45}$Ca uptake. These findings indicate that the action of ouabain is closely related to an accumulation of intracellular Na and that the possible mechanism involved is to inhibit Ca influx in response to depolarization with high-K. In the present results, it was also found that the inhibitory effects of ouabain in the taenia coli and ileum were antagonized by raising external Ca concentration. They were also associated with a decrease in cellular $^{45}$Ca content. Therefore, a possibility exists that excess cellular Na inhibits K-induced Ca influx in the ileum. The relationship between cellular Na concentration and relaxation obtained from the experimental results with the ileum also closely resembles that observed with the taenia coli. Such a possibility also exists in the portal vein and uterus since the maximal accumulation of Na in the cells attenuated their response to the high-K solution. However, the experimental results with the portal vein, aorta, and uterus were different from those with the taenia coli and ileum with regards to the inhibition of K-contraction and Ca movement by ouabain. These different results may be due to tissue-specific differences in the inhibitory effect of intracellular Na.

In contrast to the action of ouabain, verapamil caused more rapid and greater inhibition of K-contractions in all the preparations used. Furthermore, the results suggest that the order of sensitivity to verapamil is uterus $>$ portal vein $\geq$ ileum, taenia coli $>$ aorta. The effect of verapamil has been associated with a blockade of the K-induced increase in Ca influx (3-7, 26, 27). On the other hand, the dependence of muscle tension on external Ca in high-K solution was different in the various tissues, and this difference probably reflects the different sensitivities of muscles to verapamil. Finally, it is likely that the mechanisms of Ca influx for K-contractions or the utilization of the entered Ca is different in these tissues. The similarities of external Ca-dependence on K-contraction and of sensitivity to verapamil between the taenia coli and ileum reflect a similar or identical Ca translocation mechanism under the high-K depolarized condition. However, the inhibitory effect of ouabain (i.e., Na-accumulation) is incompatible with the order of the tissue-specific difference in the sensitivity to verapamil.

As another interaction of Na and Ca, the Na-Ca exchange mechanism has been established (28, 29). The existence of such a mechanism has been confirmed in the guinea-pig aorta (30, 31). With regard to an intracellular accumulation of Na, therefore, we must consider an increase in cellular Ca through an inhibition of the Na(influx)-Ca(efflux) exchange or through a facilitation of Na(efflux)-Ca(influx) exchange. In the aorta under a polarized condition, ouabain produces a sustained contraction in association with the Na accumulation. However, it has been reported that the Na-Ca exchange mechanism in the taenia coli (32-34) has little, if any, effect on contractile response as compared with the aorta. There seems to be little participation of Na-Ca exchange in the portal vein and uterus since ouabain causes only a small rise in the basal tone in the muscles of these tissues. Thus, the tissue-specific difference of the Na-Ca exchange mechanism may reflect the different effect of Na accumulation on K-contractions in the different smooth muscle tissues.
In the portal vein depolarized by high-K, ouabain transiently enhanced the developed tension. However, this increment was inhibited by the removal of external Na but not by $2 \times 10^{-6}$ M phentolamine. As the external Na-dependent increase in tension appears after ouabain-treatment, we could not detect a quantitative relationship between Na accumulation and inhibition of K-contraction in the portal vein.

Although we did not observe a potentiating effect of ouabain on K-contraction in the guinea-pig uterus, Daniel et al. (35) reported that ouabain potentiates a variety of agonist-induced contractions in the rat uterus. They have suggested that the action of ouabain may not involve a blockade of the Na, K-ATPase, but may act through adenylcyclase. If so, the presence of such a potentiating factor may apparently attenuate the inhibitory effect of ouabain in the guinea-pig uterus. However, more direct verification of these data is required.

In conclusion, ouabain inhibited K-contraction in the taenia coli and ileum of the guinea-pig, and to a lesser extent in the portal vein and the uterus, but not in the aorta. The inhibitory effect of ouabain was closely related to an accumulation of cellular Na which would cause relaxation by inhibiting K-depolarization induced Ca entry. However, it was apparent that the actions of ouabain on the different tissues were dissimilar, and the order of sensitivity is different from that of verapamil.

Acknowledgement: This study was supported in part by research grant No. 556198 from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1) van Breemen, C., Aaronson, P. and Loutzenhiser, R.: Sodium-calcium interactions in mammalian smooth muscle. Pharmacol. Rev. 30, 167–208 (1979)
2) Kishimoto, T., Ozaki, H. and Urakawa, N.: A quantitative relationship between cellular Na accumulation and relaxation produced by ouabain in the depolarized smooth muscle of guinea-pig taenia coli. Naunyn-Schmiedeberg's Arch. Pharmacol. 312, 199–207 (1980)
3) Rosenberger, L.B. and Triggle, D.J.: Ca utilization in guinea pig ileal longitudinal smooth muscle in response to a series of muscarinic agonists. Canad. J. Physiol. Pharmacol. 57, 1375–1380 (1979)
4) Bianino, G. and Johansson, B.: Effects of calcium and sodium on contracture tension in the smooth muscle of the rat portal vein. Pflügers Arch. 321, 143–156 (1970)
5) Golenhofen, K., Hermstein, N. and Lammel, E.: Membrane potential and contraction of vascular smooth muscle (portal vein) during application of noradrenaline and high potassium and selective inhibitory effects of iproveratril (verapamil). Microvasc. Res. 5, 73–80 (1973)
6) Bilek, I., Laven, R., Peiper, U. and Regnat, K.: The effect of verapamil on the response to norepinephrine or to potassium-depolarization in isolated vascular strips. Microvasc. Res. 7, 181–189 (1974)
7) Haeusler, G.: Differential effect of verapamil on excitation-contraction coupling in smooth muscle and on excitation-secretion coupling in adrenergic nerve terminals. J. Pharmacol. exp. Ther. 180, 672–682 (1972)
8) Rang, H.P.: Stimulant actions of volatile anesthetics on smooth muscle. Brit. J. Pharmacol. Chemother. 22, 356–366 (1964)
9) Friedman, S.M.: Lithium substitution and the distribution of sodium in the rat tail artery. Circulation Res. 34, 168–175 (1974)
10) Karaki, H. and Weiss, G.B.: Alteration in high and low affinity binding of $^{45}$Ca in rabbit aorta by norepinephrine and potassium after exposure to lanthanum and low temperature. J. Pharmacol. exp. Ther. 211, 86–92 (1979)
11) Kishimoto, T. and Urakawa, N.: Effects of Li-substitution on high-K induced contractions of various smooth muscle tissues in the guinea-pig. Japan. J. Pharmacol. 32, 563–572 (1982)
12) Shimizu, K., Kurosu, Y., Nakayjo, S. and Urakawa, N.: Species differences in the ouabain sensitivity of the small intestine in contractile response. Japan. J. vet. Sci. 41, 139–149 (1979)
13) Karaki, H., Ozaki, H. and Urakawa, N.: Effects of ouabain and potassium-free solution on the contraction of isolated blood vessels. Europ. J. Pharmacol. 48, 439–443 (1978)
14) Daniel, E.E.: The interconnection between active transport and contracture in uterine tissues. Canad. J. Physiol. Pharmacol. 42,
15) Godfraind, T. and Godfraind-DeBecker, A.: Actions des glucosides cardiotoniques sur le muscle lisse: I. Leur antagonisme vis-à-vis de l’histamine et de l’acetylcholine sur l’iléon isolé de cobaye. Archs int. Pharmacodyn. Thér. 144, 226-240 (1963)

16) Matthews, E.K. and Sutter, M.C.: Ouabain-induced changes in the contractile and electrical activity, potassium content, and response to drugs, of smooth muscle cells. Canad. J. Physiol. Pharmacol. 45, 509-520 (1967)

17) Griffin, J.D., Szaro, R.P. and Weltman, J.K.: Ouabain antagonism of smooth muscle contraction. J. Pharmacol. exp.Ther. 182, 378-387 (1972)

18) Broekaert, A. and Godfraind, T.: The actions of ouabain on isolated arteries. Archs int. Pharmacodyn. Thér. 203, 393-395 (1973)

19) Schatzmann, H.J. and Ackermann, H.: Die Strophanthinwirkung am Darmmuskulatur und ihre Beziehung zum Kationengehalt des Mediums. Helv. physiol. pharmacol. Acta 19, 196-213 (1961)

20) Pfaffman, M., Urakawa, N. and Holland, W.C.: Role of metabolism in K-induced tension changes in guinea pig taenia coli. Am. J. Physiol. 208, 1203-1205 (1965)

21) Pfaffman, M. and Holland, W.C.: Effects of ouabain and 2,4-dinitrophenol on Ca exchange in taenia coli. Am. J. Physiol. 211, 400-402 (1966)

22) Urakawa, N., Karaki, H. and Ikeda, M.: Effects of ouabain and metabolic inhibiting factors on Ca distribution during K-induced contracture in guinea pig taenia coli. Japan. J. Pharmacol. 20, 360-366 (1970)

23) Karaki, H., Ikeda, M. and Urakawa, N.: Effects of ouabain and 2,4-dinitrophenol on calcium distribution and exchange in guinea pig taenia coli in high-potassium solution. Japan. J. Pharmacol. 20, 530-535 (1970)

24) Bose, D.: Mechanism of mechanical inhibition of smooth muscle by ouabain. Brit. J. Pharmacol. 55, 111-116 (1975)

25) James, M.R. and Roufogalis, B.D.: The effect of ouabain on the guinea-pig ileum longitudinal smooth muscle: 2. Intracellular levels of Ca,Na, K and Mg during the ouabain response and the dependence of the response on extracellular Ca. Canad. J. Physiol. Pharmacol. 55, 1197-1203 (1977)

26) Mayer, C.J., van Breemen, C. and Casteels, R.: The action of lanthanum and D600 on the calcium exchange in the smooth muscle cells of the guinea-pig taenia coli. Pflügers Arch. 337, 333-350 (1972)

27) Riemer, J., Dörfler, F., Mayer, C.J. and Ulbrecht, G.: Calcium-antagonistic effects on the spontaneous activity of guinea-pig taenia coli. Pflügers Arch. 351, 241-258 (1974)

28) Reuter, H. and Seitz, N.: The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. J. Physiol. 195, 451-470 (1968)

29) Baker, P.F., Blaustein, M.P., Hodgkin, A.L. and Steinhardt, R.A.: The influence of calcium on sodium efflux in squid axons. J. Physiol. 200, 431-458 (1969)

30) Ozaki, H., Karaki, H. and Urakawa, N.: Possible role of Na-Ca exchange mechanism in the contractions induced in guinea pig aorta by potassium free solution and ouabain. Naunyn-Schmiedeberg’s Arch. Pharmacol. 304, 203-209 (1978)

31) Ozaki, H. and Urakawa, N.: Na-Ca exchange and tension development in guinea-pig aorta. Naunyn-Schmiedeberg’s Arch. Pharmacol. 309, 171-178 (1979)

32) Raeymaekers, L., Wuytack, F. and Casteels, R.: Na-Ca exchange in taenia coli of the guinea-pig. Pflügers Arch. 347, 329-340 (1974)

33) Casteels, R. and van Breemen, C.: Active and passive Ca²⁺ fluxes across cell membranes of the guinea-pig taenia coli. Pflügers Arch. 359, 187-207 (1975)

34) Brading, A.F.: Calcium-induced increase in membrane permeability in the guinea-pig taenia coli: Evidence for involvement of a sodium-calcium exchange mechanism. J. Physiol. 275, 65-84 (1978)

35) Daniel, E.E., Massingham, R. and Nasmyth, P.A.: An action of ouabain to promote smooth muscle contraction unrelated to membrane ATPase inhibition. Brit. J. Pharmacol. 34, 231P-232P (1968)