Species Differences in the Inhibitory Effect of Ouabain on High 
K-Induced Contraction in the Ileal Longitudinal Muscle

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Abstract—Effects of ouabain on a high K-induced contraction and intracellular 
Na and K contents of the ileal longitudinal muscle preparations isolated from various 
animal species was compared. In muscles of various animals (monkey, dog, cat, 
rabbit, guinea-pig, rat and mouse), the high K-induced contraction was inhibited 
by verapamil (5×10⁻⁸ M) or ouabain (3×10⁻⁶ M), and both the inhibitions were 
antagonized by an increase in external Ca in a competitive manner. Species 
differences were shown in the ouabain effect but not in the verapamil one. Regarding 
the sensitivity to ouabain, the muscles were divided into two groups, that is, the 
muscle from monkey, rabbit, guinea-pig or dog belongs to the high sensitivity group, 
and that from cat, rat or mouse belongs to the low one. The order of sensitivities 
of the muscles to ouabain in the relaxation was consistent with that in Na,K-ATPase 
inhibition, as reported by Repke et al. (1965). Intracellular Na contents of muscles 
were increased by an addition of ouabain to the high K solution, and the rate and 
amount of the accumulation of intracellular Na varied in these muscles. Except 
for the cat muscle, a high correlation was noted between the ouabain-induced 
relaxation and the amount of intracellular Na accumulation. However, the regression 
coefficients were variable: 4.1 in the monkey muscle, 2.2 in the rat one, and about 1.0 
in the others. That is, the monkey muscle showing the high regression coefficient 
value probably has a high sensitivity to the inhibition of intracellular Na in the high 
K-induced contraction. In summary, the high K-induced contractions of ileal 
longitudinal muscle preparations isolated from the various animals species were 
inhibited by ouabain (10⁻⁶–10⁻⁴ M), in varying degrees. It is suggested that the 
species difference in the ouabain-induced relaxation is composed of two species 
differences in the inhibition of Na,K-ATPase in the muscle and the sensitivity of the 
muscles to the accumulated sodium and that the ouabain-induced relaxation in 
the ileal muscles is probably induced by an inhibition of accumulated Na to the 
increased Ca influx by depolarization.

Ouabain, a specific inhibitor of Na,K- 
ATPase, has an inhibitory action on the Na 
pump of the cell, causing intracellular Na 
accumulation (1). It was reported that 
ouabain inhibited a sustained contraction of 
the smooth muscle of guinea-pig taenia coli 
in a solution containing potassium at a high 
concentration (high K solution) (2–5). Fur 
thermore, it was noted that a high correlation 
 existed between the ouabain-induced re- 
laxation and the Na accumulation in taenia 
coli (6–8). A mechanism of the ouabain-
induced relaxation has been proposed that 
the intracellular Na accumulation suppressed 
the increased Ca influx caused by depolar-
ization (7, 8).

On the other hand, it was known that 
ouabain induced a contraction through 
depolarization caused by an inhibition of the 
electrogenic Na pump in taenia coli (9, 10),
Shimizu et al. (11) has found that there were species differences in the ouabain-induced contraction in the ileum and suggested that the difference is probably due to species differences in the inhibition of Na,K-ATPase by ouabain.

In the present study, we compared the effects of ouabain on tension and intracellular Na and K contents of ileal longitudinal smooth muscle preparations isolated from various animal species (monkey, dog, cat, rabbit, guinea-pig, rat and mouse), in the high K solution.

**Materials and Methods**

The experimental animals used were crab eating monkeys (*Macaca fascicularis*), weighing 3–5 kg, dogs (about 10 kg), cats (about 2 kg), rabbits (3–4 kg), guinea-pigs (300–400 g), rats (250–350 g), and mice (20–25 g). All mammalia were sacrificed by a blow on the neck. After exsanguination, the abdomen was opened, and the lower part of the ileum, to 5 cm in distance from opening of the caecum, was discarded from the ileal sample. The ileal longitudinal muscle preparations were made as described by Paton and Aboo Zar (12). One end of the strips was bound to a glass holder with nylon thread, and the other end was connected with the thread to a strain gauge transducer (Nihon Kohden). The muscle was suspended in an organ bath containing 15 ml of physiological salt solution (PSS) and equilibrated for 60 min. The muscle tension was recorded isometrically. The PSS employed was a modified Tyrode's solution of the following composition (mM): NaCl, 136.8; KCl, 5.4; CaCl₂, 2.5; MgCl₂, 1.0; NaHCO₃, 11.9 and glucose, 5.5. The solution was aerated with a 95% O₂-5% CO₂ gas mixture at 37°C and pH 7.2.

The tension loaded to the muscle strips was about 0.5 g. Sixty mM KCl was hyperosmotically applied twice to the muscle after the 60 min incubation in PSS. The maximal tension of the tonic contraction induced by the high K solution (65.4 mM) was regarded as a reference response (100%). In some experiments, the effect of the drug on the contraction induced by adding Ca to the muscles incubated in Ca-free high K solution (Ca-induced contraction) was investigated. Ca-free solution was made by omitting CaCl₂ from the original medium without any substitution for osmolality. An elevation of Ca concentration above 10 mM was made by substituting Tris-HCl for NaHCO₃ in the PSS, and its solution was bubbled with 100% O₂ gas.

Intracellular Na and K contents were estimated as reported by van Breemen et al. (13). After incubation with a test solution, the muscle was exposed to a La-solution (294.9 mM sucrose, 1.0 mM LaCl₃, 11.9 mM Tris-HCl) at 0.5°C. 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 dissolved in 0.01 N HCl solution. CsCl (1 g/l) was added to standard and diluted solutions to inhibit mutual interference of Na and K. Ion concentration of the diluted sample was measured using a flame photometer (Varian, Type AA-275).

The drugs used were ouabain (Merck), verapamil (Eisai), atropine sulphate (Sigma) and tetrodotoxin (Sankyo).

**Results**

1. The high K-induced contractions of the ileal longitudinal muscles isolated from various animal species

When high K solution was hyperosmotically applied to the muscles isolated from various animals, it caused rapid phasic contractions followed by sustained ones in the muscles, which were maintained at a steady level during 120 min. Since these high K-induced contractions were not affected by treatments with 10⁻⁶ M atropine and 10⁻⁷ g/ml tetrodotoxin, it seems that the high K-induced contractions may not be mediated by released nerve transmitters.

In the next series of experiments, the effect of verapamil, an organic Ca antagonist, was examined on the high K-induced contraction of the ileal muscles. Thirty minutes after the application of high K solution, verapamil (10⁻⁹–3×10⁻⁷ M) was added cumulatively. In the muscles from all the species, the high K-induced contraction was completely inhibited by verapamil (3×10⁻⁷...
Further, verapamil produced a concentration-dependent relaxation in the high K-induced contraction in the muscle of cat, rabbit, guinea-pig or rat. The concentration of verapamil which inhibited the contraction by 50% (IC50) in the cat, rabbit, rat or guinea-pig was $1.4 \times 10^{-8}$ M, $1.5 \times 10^{-8}$ M, $1.5 \times 10^{-8}$ M or $4.0 \times 10^{-8}$ M, respectively. These IC50 values of verapamil were in a narrow range of concentration from $1.5 \times 10^{-8}$ M to $4.0 \times 10^{-8}$ M, and it is difficult to find species differences in the verapamil inhibition of the high K-induced contraction.

2. Effect of ouabain on high K-induced contraction

Monkey, dog, rabbit and guinea-pig: Thirty minutes after an application of the high K solution, ouabain ($10^{-6}$ M–$10^{-4}$ M) was applied to the muscle preparations from the four kinds of animals. In the muscle of monkey or dog, an application of ouabain at a concentration of $10^{-6}$ M gradually decreased the tension developed by high K solution. The developed tension of the rabbit or guinea-pig muscle was not changed by $10^{-6}$ M ouabain, but was decreased by $10^{-5}$ M ouabain, and further inhibited by $10^{-4}$ M ouabain.

Rat and mouse: In the muscle of rat or mouse, the high K-induced contraction was not changed by an application of $10^{-5}$ M ouabain, but slightly inhibited by $10^{-4}$ M ouabain and much more inhibited by $10^{-3}$ M ouabain.

Cat: When $10^{-5}$ M ouabain was applied to the cat muscle, the high K-induced contraction was transiently enhanced, then gradually declined to a steady level similar to that maintained by high K solution in the absence of ouabain for 2 hr. An application of ouabain at a higher concentration of $10^{-4}$ M did not enhance the high K-induced contraction, but gradually decreased it within 60 min after the application.

Figure 1 shows the effect of ouabain ($10^{-5}$ M) on the high K-induced contraction in the muscles from the seven animal species.

From these results, the inhibitory effect of ouabain on the high K-induced contraction was divided two groups. That is, the muscles of monkey, rabbit, guinea-pig and dog belong to a high sensitivity group, and the muscles of rat, mouse and cat to a low sensitivity one. In cat muscle, ouabain ($10^{-5}$ M) transiently potentiated the high K-induced contraction followed by a gradual decline. It can be said that there were species differences in the ouabain-induced relaxation of the depolarized ileal muscles.

3. Changes in intracellular Na and K contents induced by ouabain

In the muscles of all species, the intracellular Na and K contents in high K solution were determined with time. Both the contents were not significantly different from those in normal solution at 120 min. Ouabain at

![Figure 1](image_url)
concentrations from $10^{-6}$ M to $10^{-4}$ M was applied to the muscles 30 min after an incubation in the high K solution. The drug increased the intracellular Na contents with time and in a concentration-dependent manner. On the contrary, ouabain decreased intracellular K contents of muscles.

The correspondence was shown in all the muscles except the rat or mouse one. Changes of intracellular Na and K contents induced by $10^{-5}$ M ouabain are shown in Fig. 2-A, B. As shown in Fig. 2-A, the rates and amounts of accumulation of Na induced by ouabain were variable in the muscles. The intracellular Na content after 120 min incubation in the high K solution with $10^{-5}$ M ouabain was 75, 57, 55, 53, 27, 15 or 13 mmole/kg wet wt. in the muscle isolated from rabbit, guinea-pig, dog, cat, monkey, rat or mouse, respectively. From these results,

Fig. 2. Effects of ouabain on the intracellular Na (A) and K (B) contents in high K-depolarized preparations of the ileal longitudinal muscles from various animals. Ordinate: intracellular Na or K content (mmole/kg wet wt.). Abscissa: time (min) after application of $10^{-5}$ M ouabain. Given are the mean values±S.E.M. for 6 determinations.
ouabain induced more rapid and larger increase in intracellular Na content in the rabbit, dog, guinea-pig or cat than that in the monkey. The order of the ouabain-induced relaxation corresponded with that of Na accumulation by ouabain in the muscles of the animal species except for cat and monkey. Moreover, a high and negative correlation between the developed tension and the amount of intracellular Na accumulation was shown in the muscle of all the species except for the cat, in the high K solution with ouabain (10⁻⁶–10⁻⁴ M) (Fig. 3). The values of the regression coefficient in the muscle of rabbit, guinea-pig, dog, rat and monkey were 1.0, 1.0, 1.3, 2.2 and 4.1, respectively. The value of the regression coefficient seems to exhibit a muscle sensitivity to intracellular Na inhibiting the high K-induced contraction. In the smooth muscle of the guinea-pig taenia coli, the regression coefficient was calculated from the data by Kishimoto et al. (8). The value of taenia coli, 2.0, is larger than that of the ileal longitudinal muscle in guinea-pig, 1.0. It can be said that there are not only species differences in the muscle sensitivity to Na but also portion differences in the intestinal smooth muscles. 4. The effects of verapamil and ouabain in the Ca-induced contractions

It is generally accepted that a Ca-induced contraction of depolarized muscle is regarded as an experimental model for Ca influx from the extracellular medium. In a series of experiments, effects of verapamil and ouabain were studied on the Ca-induced contraction in the rabbit and guinea-pig muscles. Muscles of both the animals were incubated for 15 min in a high K solution omitting Ca ions, then a contraction was induced by cumulatively adding Ca (0.25–10.0 mM) to the medium in a concentration-dependent manner. In both the muscles, a pretreatment with verapamil (1×10⁻¹¹–5×10⁻¹¹ M) for 15 min showed a parallel shift of the concentration-response curve of Ca to the right, exhibiting an antagonistic action. As the dose of verapamil was increased, the degree of the shift also increased. By a cumulative addition of Ca after preincubation with ouabain (3×10⁻⁶ M) for 90 min, the concentration-response curve of Ca also showed a parallel shift to the right (Fig. 4-A, B). Since further increase of the Ca concentration (10–20 mM) in the Tris buffered solution caused a maximum contraction even in the presence of verapamil or ouabain, these actions are probably regarded as a competitive antagonistic pattern (Fig. 4-A′, B′). From the data, it is assumed that ouabain

**Fig. 3.** Relationship between developed tension and intracellular Na content of ileal longitudinal muscles isolated from various animals. The muscles were soaked in the high K solution without or with ouabain for 120 min. Regression coefficient and correlation index (γ) are indicated in the upperportion.
as well as verapamil competitively inhibit Ca influx across the membrane suppressing the contraction in the depolarized smooth muscle cells.

Fig. 4. Effects of verapamil and ouabain on the Ca-induced contraction in the ileal longitudinal muscle isolated from rabbit and guinea-pig. The muscle was incubated in Ca-free, high K solution for 2 hr; then an appropriate concentration of Ca was added cumulatively to the bicarbonate or Tris-HCl-buffered medium. Relative tension is expressed as percent of the hyper-65K contraction (Ca, 2.5 mM) which is plotted on the ordinate as a function of external Ca concentration on the abscissa. The preparations were pretreated with verapamil for 15 min or with ouabain for 90 min. Given are the mean values±S.E.M. as vertical bars of 6–12 experiments. Bicarbonate-buffered medium: rabbit muscle (A), guinea-pig (B) Tris-HCl-buffered medium: rabbit muscle (A'), guinea-pig (B').
Discussion

In the present data, verapamil or ouabain inhibited the sustained contraction induced by the high K solution in the ileal longitudinal muscle preparation isolated from various animal species in the same manner as in the guinea-pig taenia coli. The inhibitory effect of verapamil on the contraction were almost similar in degree in all the muscles. However, in the case of ouabain treatment, the muscle from monkey, rabbit, guinea-pig or dog had higher sensitivity to ouabain, but that from rat, mouse or cat had lower sensitivity. Moreover, the increase in intracellular Na content by ouabain in the high concentration varied in the muscles from all the animal species. Except for the muscles of cat and monkey, the order of species differences in sensitivity of the muscles to ouabain in the inhibition of the high K-induced contraction was not only similar to that in Na accumu-
lation by ouabain into the muscles, but
similar to those in the ileal contractile response
to ouabain (11) and in the inhibition to Na,K-
ATPase of cardiac muscles (14).

Except for the muscle of cat, high cor-
relation was noted between the inhibition of
high K-induced contraction and the amount
of intracellular Na accumulation. In the
monkey muscle, the inhibition of ouabain to
high K-induced contraction was large,
though the accumulated Na content was
small. The regression coefficient in the monkey
muscle, 4.1, was larger than those in the dog,
rabbit or guinea-pig muscle, 1.0 as shown in
Fig. 3. That is, the inhibitory action of intra-
cellularly accumulated Na in the monkey
muscle is probably much more effective than
those in other muscles on the high K-
induced contraction. On the other hand, it is
unclear how the regression coefficient in the
rat muscle, 2.2, is involved in the species
difference in the ouabain-induced relaxation.
From these data, it is assumed that the species
difference in the ouabain-induced relaxation
is composed of two kinds of species differ-
ences in the inhibition by ouabain of Na,K-
ATPase in the muscles and in the sensitivity
of the muscle to the accumulated sodium.

In the muscles of cat, 10⁻⁴ M ouabain
slightly inhibited the high K-induced con-
traction and increased the intracellular Na
content; however, the lower concentration of
ouabain (10⁻⁶–10⁻⁵ M) failed to inhibit
and potentiated the contraction, although it
increased the Na content. Kishimoto and
Urakawa (8) reported that ouabain failed to
induce a relaxation, but slightly increased the
intracellular Na content in the guinea-pig
aorta and portal vein. Accordingly, the at-
titudes of the cat ileal muscle to the ouabain-
induced relaxation and intracellular Na
accumulation by ouabain seem to be almost
similar to those in the guinea-pig vascular
smooth muscles.

It is known that the high K-induced con-
traction is induced by increase in Ca influx
across the membrane in guinea-pig taenia
coli (15–17). Further, it was reported that
the ouabain-induced relaxation was closely
related to an accumulation of cellular Na,
antagonized by increasing external Ca ion,
and also involved in the inhibition in ⁴⁵Ca
uptake. From these observations, it was sug-
gested that the accumulated Na inhibits the
increased Ca influx in the depolarized muscle
(7). A Ca entry stimulater, CGP28392 (4-[2-
(difluromethoxy)-phenyl]-1,4,5,7-tetra-
hydro-2-methyl-5-oxofuro[3,4-b]pyridine-3-
carboxylic acid ethylester) markedly an-
tagonized the inhibitory effect of verapamil
on the extra Ca entry into the depolarized
smooth muscle of guinea-pig taenia coli, but
not the inhibitory effect of ouabain or
accumulated Na on it (Ozaki and Urakawa,
unpublished data). This data suggests that
the action site of verapamil on the voltage-
dependent Ca channel is not the same as that
of accumulated Na on it. In the present data,
the high K-induced contractions in the ileal
longitudinal muscles prepared from various
animals were dependent on Ca ion in the
external medium and inhibited by verapamil
in the manner of competitive antagonism.
The data suggests that the high K-induced
contraction in the ileum as well as taenia
coli is also caused by an increased Ca influx
caused by depolarization. The ouabain-
induced relaxation was also antagonized by
the increase in external Ca in a competitive
manner. Moreover, a high correlation existed
between the increase in intracellular Na and
ouabain-induced relaxation. These data in the
ileal longitudinal muscle is probably consist-
ent with the proposal that intracellular Na
accumulation inhibits Ca influx in the
depolarized muscle of taenia coli (7).

In summary, the high K-induced con-
tractions of the ileal longitudinal muscle
preparations from various animals were
inhibited by ouabain (10⁻⁶–10⁻⁴ M). It is
suggested that the species difference in the
ouabain-induced relaxation is composed of
two species differences in the inhibition by
ouabain of Na,K-ATPase of the muscles and
in the sensitivity of the muscle to the ac-
cumulated sodium, and the ouabain-induced
relaxation in the ileal longitudinal muscle as
well as taenia coli is probably induced by the
inhibition of accumulated Na on the increased
Ca influx by depolarization.

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