EFFECTS OF OUABAIN AND 2, 4-DINITROPHENOL ON CALCIUM DISTRIBUTION AND EXCHANGE IN GUINEA PIG TAENIA COLI IN HIGH-POTASSIUM SOLUTION

HIDEAKI KARAKI, MIYOSHI IKEDA AND NORIMOTO URAKAWA

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

Received for publication May 16, 1970

Previous studies have shown that Ca influx increased but Ca efflux did not change during the contracture of taenia coli induced in hypertonicity added 40 mM potassium solution (1) and the results were supported by the others (2-6). Further, the movements of Ca in the muscle in high-K solution might be differently affected by ouabain and 2, 4-dinitrophenol during the abolition of the tonic tension by them (6, 7).

The experiments reported here were carried out in purpose to find the nature of Ca distribution and exchange in guinea pig taenia coli in high-K solution using ouabain and 2, 4-dinitrophenol.

METHODS

Strips of taenia coli isolated from white male guinea pigs were suspended in an organ bath containing Tyrode solution of the following composition (mM): NaCl 136.8, KCl 2.7, CaCl₂ 2.5, MgCl₂ 1.0, NaH₂PO₄ 0.4, NaHCO₃ 11.9 and glucose 5.5, saturated with 95% O₂ and 5% CO₂ mixture at 37°C. Isotonically added 40 mM K solution (iso-40 K-S.) was made by replacing 40 mM NaCl in Tyrode solution with equimolar KCl. Tension changes were isometrically recorded with a mechanoelectro-transducer.

⁴⁺Ca uptake: After pretreatment, muscle strips were soaked in iso-40 K-S. for 30 minutes, then 2.5 × 10⁻⁴ M ouabain or 1 × 10⁻⁴ M 2, 4-dinitrophenol (DNP) was added. Thirty minutes after the addition of ouabain or DNP, corresponding to zero time of Fig. 1, ⁴⁺Ca (1 × 10⁶ counts/min·ml) was added to the medium for incubation. After 5, 15 or 30 minutes incubation period, the strips were removed from the bath and radioactivities in the muscle strips were counted (1, 4).

Tissue Ca: Strips were treated in the same manner as that in the preceding paragraph except that ⁴⁺Ca was not used and Ca content of the tissue was determined using atomic absorption spectrophotometer (1, 4).

⁴⁺Ca efflux: Muscle strips were incubated in iso-40 K-S. with ⁴⁺Ca (200 μCi/ml) for 180 to 200 minutes and then washed by dipping into successive tubes containing iso-
40 K-S. with or without ouabain or DNP. After the wash out period, radioactivities remaining in each tube and muscle were counted (1).

**Tightly bound fraction**: The tissue Ca fraction which did not exchange within 4 minutes was tentatively designated as "tightly bound fraction" (TBF) (1), which was determined by the same procedure as \(^{45}\text{Ca}\) uptake experiment except that muscle strips were washed three times in 4 minutes at the end of each incubation period with non-radioactive solution of the same composition as incubation one.

**Extracellular space**: Extracellular space (ECS) was measured using \(^{14}\text{C}\)-sorbitol by the same time schedule as \(^{45}\text{Ca}\) uptake experiment (6).

**RESULTS**

*Iso-40 K-S.* induced a tonic tension development averaging about 10 g in taenia coli and the tension was abolished by \(2.5 \times 10^{-6}\) M ouabain within 30 minutes and by \(1 \times 10^{-4}\) M DNP within 10 minutes.

\(^{45}\text{Ca}\) uptake of taenia coli from normal Tyrode solution, *iso-40 K-S.*, *iso-40 K-S.* with ouabain or with DNP is illustrated in Fig. 1. Tissue Ca and TBF of the muscle in these media are also shown. Size of ECS, measured with \(^{14}\text{C}\)-sorbitol, was 40.0±0.1% (8) in normal medium and did not change significantly in the media employed (\(P<0.01\)).

It can be seen from Fig. 1 that, in *iso-40 K-S.*, the muscle strips took up 4.86 mEq/kg of Ca from the solution within 5 minutes while 3.46 mEq/kg in normal one. Tissue Ca and TBF also increased in *iso-40 K-S.* compared with control ones. In the presence of ouabain, the increased rate of \(^{45}\text{Ca}\) uptake and tissue Ca remained high but TBF de-

---

**Fig. 1.** Tissue Ca(□), \(^{45}\text{Ca}\) uptake (○) and TBF (△) of taenia coli.
A : Normal Tyrode solution (filled symbols) and *iso-40 K-S.* (open symbols).
B : *Iso-40 K-S.* with \(2.5 \times 10^{-6}\) M ouabain.
C : *Iso-40 K-S.* with \(1 \times 10^{-4}\) M DNP.
Each value is the mean of 8 to 12 experiments and ±SE is shown.
creased to the control level. In the presence of DNP, in contrast with ouabain, 45Ca uptake, tissue Ca and TBF decreased to the control levels. It is also shown that tissue Ca mostly exchanged within 15 minutes in these environments.

Fig. 2 shows the loss of 45Ca, first into iso-40 K-S., and 100 minutes after the beginning of wash out, into iso-40 K-S. with ouabain or DNP. The rate of loss of 45Ca, shown by the lower curve in Fig 2, slightly increased after the addition of ouabain or DNP, but the effect was very small and not always noticeable in the upper curve showing the amount of 45Ca remaining in the muscle.

![Diagram](image)

**Fig. 2. Loss of 45Ca from taenia coli.**

Abscissa, time after beginning of wash out. Logarithmic ordinate, the amount of radioactivity in the muscle (counts/min) as a fraction of the initial amount (upper) and the rate of 45Ca efflux (counts/min) as a fraction of the initial rate (lower).

Muscle was washed in iso-40 K-S. during first 100 minutes and then in iso-40 K-S. with $2.5 \times 10^{-4}$M ouabain (left) or with $1 \times 10^{-4}$M DNP (right). The same result was obtained in three other pieces of taenia coli.

It is summarized that 45Ca uptake and tissue Ca of the muscle increased in iso-40 K-S. and the increments were not affected by ouabain but decreased by DNP. Iso-40 K-S. also increased TBF, which was abolished by both ouabain and DNP. Loss of 45Ca into iso-40 K-S. scarcely altered in the presence of ouabain or DNP.

**DISCUSSION**

Hypertotonically added 40 mM K solution induces a contracture in guinea pig taenia coli which is separable into two components, phasic and tonic ones (2). It has been suggested that in the phasic contraction, sufficient Ca is released from a cellular site to
initiate contraction, whereas in the tonic contraction enough Ca crosses the membrane (1, 2) and Ca moves towards the "tightly bound fraction" (TBF) during both responses (1, 2). The present and the preceding data (6) showed that the movement and distribution of Ca in taenia coli during tonic response induced in isotonically added 40 mm K solution were almost the same as those in hypertonically added 40 mm K solution.

The rate of loss of Ca from taenia coli has been shown to be largely controlled by a physical process although the possibility of a slight participation of metabolism-dependent Ca-exclusion mechanism could not be excluded (3, 8), and the present data seem to support the suggestion. On the other hand, Plaffman and Holland (7) reported that the increase in tissue Ca in hypertonically added 40 mm K solution did not change in the presence of DNP, but was decreased by ouabain, and suggested the existence of DNP-sensitive Ca-extrusion pump in taenia coli. However, Urakawa et al. (6) reported and discussed the contradictory results which are confirmed by the data in this report.

In the present observations the increase in both Ca influx and tissue Ca of the muscle in high-K solution were abolished only by DNP and the increase in TBF was abolished by ouabain or DNP. Further, the effects of lithium substitution for sodium on tissue Ca and TBF were similar to those by ouabain, and the effects of glucose removal and anoxia on them were similar to those by DNP in high-K solution (6). These data suggest that the influx of Ca and the movement of Ca towards TBF are active processes possibly related to energy-rich phosphate compounds and the latter process might have a concern with Na, K-ATPase, the existence of which (9) and the possible relationship with ouabain (10) in taenia coli have been reported. It is also suggested that if there were a Na-Ca linked transport system in taenia coli, as reported in other tissues (11-14), it would be at TBF, the size of which is closely related to the tension development of taenia coli (1, 2, 4-6).

Recently, oxygen consumption of taenia coli was reported to increase in iso-40 K-S. (15) which was not affected by ouabain but decreased by the removal of external Ca (16) and it was suggested that the increase in oxygen consumption might have a correlation with the increase in the intracellular Ca (6, 17). In this report, it was demonstrated that, although TBF decreased, Ca influx and efflux did not change in iso-40 K-S. with ouabain. Therefore, the former suggestion (6, 17) should be revised as; the increase in oxygen consumption might have a correlation with the increase in Ca influx and/or the increase in the movement of intracellular Ca other than TBF.

Evidences on the metabolism-dependent Ca entry have been reported in this communication and in others (1, 2, 4-6) in the presence of high concentration of potassium. The presence of metabolism dependent Ca exclusion mechanism has also been proposed in taenia coli (3, 18) and in rat uterine muscle (19) in the normal physiological saline solution. These two mechanisms do not invariably contradict each other as it might be possible that, in polarized state, the Ca exclusion mechanism exists and when depolarized by high-K, Ca is taken up actively overcoming the barrier of the exclusion mechanism.
SUMMARY

1. Effects of ouabain and 2, 4-dinitrophenol (DNP) on Ca distribution and exchange in guinea pig taenia coli in isotonically added 40 mm K solution were investigated.

2. During the contracture induced in 40 mm K solution, 45Ca uptake, tissue Ca and Ca fraction which did not exchange within 4 minutes (tightly bound fraction, TBF) increased. Ouabain (2.5 x 10^-6 M) and DNP (1 x 10^-4 M) abolished the tonic tension development and the increase in TBF in 40 mm K solution. The increase in both 45Ca uptake and tissue Ca was not affected by ouabain but decreased by DNP. 45Ca efflux and extracellular space were scarcely influenced by them.

3. From these data, it is suggested that the net influx and the movement of Ca towards TBF are active processes possibly related to energy-rich phosphate compounds and the latter process might have a concern with Na, K-ATPase.

APPENDIX

In estrogen-dominated rat myometrium, Krejci and Daniel (20) have recently reported that 45Ca efflux was slowed in the presence of isotonic or isometric contraction of the smooth muscle induced by raising external K concentration (isotonic, 44 mm or 119 mm) or addition of oxytocin at 27°C, and the efflux was accelerated when relaxation occurred during efflux experiment. As efflux of extracellular 14C-sucrose was also slowed in contracted muscle, they concluded that contraction slows diffusion of substances in the extracellular space of the uterine muscle, and that comparison of contracted to relaxed uterine tissue is inappropriate because of weight changes during contraction, and because efflux of tracer through the extracellular fluid is altered by contraction itself, and suggested that earlier studies on calcium fluxes in other smooth muscles should be reconsidered in light of their experiments.

In the present data using taenia coli at 37°C, tissue Ca and 45Ca uptake increased in muscles either contracted in high-K solution or relaxed in high-K solution with ouabain, compared to those in muscle in normal solution, suggesting that the change in Ca movement might separate from the sequence of mechanical event accompanying the contraction. Size of TBF, which increased only in contracted taenia (1, 2, 4-6), might be also not the result of the change in extracellular space by contraction as the size of TBF was determined by rinsing the incubated muscle for 4 minutes (see Methods) and the most part of extracellular space would be cleared. Further, the increase in the size of TBF in contracted muscle by high-K was too large to be attributed to the change in extracellular space.

It is difficult to compare the data by Krejci and Daniel (20) and ours because of the difference in experimental conditions and materials except to say that the change in Ca movement in taenia coli induced by high-K could not be explained by the change in extracellular space as suggested by Krejci and Daniel (20).
REFERENCES

2) URAKAWA, N. AND HOLLAND, W.C.: Am. J. Physiol. 207, 873 (1964)
3) GOODFORD, P.J.: J. Physiol. 176, 180 (1965)
8) BAUER, H., GOODFORD, P.J. AND HÜTER, J.: J. Physiol. 176, 163 (1965)
17) URAKAWA, N., IKEDA, M., SAIITO, Y. AND SAKAI, Y.: Jap. J. Pharmac. 18, 500 (1968)