⁴⁵Ca UPTAKE AND TISSUE Ca OF GUINEA PIG TAENIA COLI IN ISOTONIC HIGH-K/Na-DEFICIENT MEDIUM

NORIMOTO URAKAWA, HIDEAKI KARAKI

AND MIYOSHI IKEDA

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

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It has already been reported that ⁴⁵Ca uptake and total Ca content of guinea pig taenia coli increased when 40 mM K was added hypertonically to the external medium (1, 2). However, the addition of high-K isotonically, that is, an appropriate concentration of external sodium replaced by potassium, to depolarize and/or contract smooth muscle preparations, is the method that is widely employed.

We have reported (3) that in isotonic high-K medium, which contained less Na, smooth muscle of taenia coli showed a shape of contracture different to that shown in hypertonic 40 mM K medium, which contained normal concentration of Na.

In the present paper, changes in ⁴⁵Ca uptake and tissue Ca of guinea pig taenia coli in isotonic 152 mM K/Na deficient medium were studied and the results were compared with those in hypertonic 40 mM K medium.

METHODS

Strips of taenia coli isolated from 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 gas mixture at 37°C. Tension changes were recorded isometrically with Nihon Kohden multi-purpose polygraph.

⁴⁵Ca uptake: After pretreatment, muscle strips were placed in an incubation medium containing ⁴⁵Ca (1 × 10⁶ counts/minute·ml) for specified intervals with and without elevated K. At the end of each incubation period, the strips were washed three times in 4 minutes with a non-radioactive Tyrode solution of the same composition as the incubation medium, removed from the bath, blotted gently on filter paper and weighed. They were then transferred to quartz tubes to which 0.1 ml HClO₄ was added. The samples were ashed in a furnace at 550°C, diluted with 0.1 N HCl, pipetted into planchettes, dried and radioactivity was counted in a gas flow counter (Aloka window type Model PS-13). Data are expressed as counts per minute per kilogram wet weight of muscle. Radioactive ⁴⁵Ca was supplied by Nuclear Science and Engineering Corp., U.S.A., as the chloride.

浦川 紀元・唐木 英明・池田 三義

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Tissue Ca: The time schedule and conditions in the experiments in which tissue Ca was determined were the same as in isotope studies, except that a solution containing 0.2% SrCl_2 and 0.4% disodium ethylenediaminetetraacetate (EDTA) was used for dilution of the ashed samples to minimize the interferences from ions other than Ca (4). Ca content of the solution was determined by Perkin-Elmer Model 303 atomic absorption spectrophotometer. Tissue Ca data are expressed as milliequivalents per kilogram wet tissue uncorrected for extracellular space.

High-K medium: Hypertonic 40 mM K medium (*hyper-40 K medium*) was made by adding KCl crystals to normal Tyrode solution to make a final concentration of 42.7 mM KCl. Composition of isotonic high-K medium (*iso-152 K medium*) was as follows (mM): KCl, 139.5; CaCl_2 , 2.5; MgCl_2 , 1.0; KH_2PO_4 , 0.4; KHCO_3 , 11.9; and glucose, 5.5.

RESULTS

Tension changes induced by hypertonic and isotonic high-K media

Tension change curves obtained by soaking strips of taenia coli in *hyper-40 K* and *iso-152 K* media are shown in Fig. 1. In *hyper-40 K* medium, the muscle strips developed a sharp rise in tension followed by a steady tension level averaging 12 g. In *iso-152 K* medium, the sharp rise in tension was followed by a gradual decline and within 20 minutes it reached a steady level of about 2 g.

K-induced contracture in *hyper-40 K* medium was affected neither by 1×10^{-7} g/ml hyoscine (5) nor by 1×10^{-5} M tetrodotoxin, which is known to block the nerve conductance, suggesting that this K-induced contracture is independent of any nervous influence. From these data, the difference between contracture curves induced by *hyper-40 K* and *iso-152 K* media in the absence of external sodium is thought to be caused by something other than a block of nerve function.

FIG. 1. Tension change curves produced by *hyper-40 K* and *iso-152 K* media in guinea pig taenia coli.

Hyper-40 K medium contained 42.7 mM K and 149.1 mM Na and *iso-152 K* medium contained 151.8 mM K and 0 mM Na.

⁴⁵Ca uptake and tissue Ca of taenia coli in hypertonic and isotonic high-K media

⁴⁵Ca uptake and total Ca content of taenia coli incubated in *iso*-152 K medium for 15 and 30 minutes are shown in Fig. 2. Results in normal and *hyper*-40 K media are also shown. Ca content of the muscle in *hyper*-40 K medium was significantly higher than control value ($P < 0.01$) and that in *iso*-152 K medium was significantly lower than that ($P < 0.01$). ⁴⁵Ca uptake of the muscle also significantly increased in *hyper*-40 K medium ($P < 0.01$) but did not increase in *iso*-152 K medium.

DISCUSSION

In the present data, it is demonstrated that not only tension change curves but also Ca movements of guinea pig taenia coli differ in *hyper*-40 K and *iso*-152 K media, that is, in *hyper*-40 K medium, large tonic tensions averaging 12 g were induced and both ⁴⁵Ca uptake and total Ca content of the muscle increased, and in *iso*-152 K medium, only a small tonic tension of about 2 g remained. Further, ⁴⁵Ca uptake showed little or no change although total Ca content decreased considerably.

Urakawa and Holland (1) reported the increase in both ⁴⁵Ca uptake and total Ca content of guinea pig taenia coli in hypertonic 40 mM K medium. Van Breeman and Daniel (6), however, found a decrease in ⁴⁵Ca uptake in rat uterine smooth muscle in high-K/low-Na medium. This difference in results has been discussed by Van Breeman and Daniel (6) as being due to a difference in experimental plannings, that is, within 4 minute-wash out period after ⁴⁵Ca uptake experiments, employed by Urakawa and Holland (1), almost 90% of the tissue Ca exchanges and residual ⁴⁵Ca could have resulted from a slower efflux during the wash period. As for the total Ca gain, they (6) also discussed that it could have been caused by a squeezing out of extracellular fluid, which had a lower concentration of Ca (1.8 mM) than normal Ca content of the cells, with a resulting increase in total Ca content during contracture induced by hypertonic 40 mM K medium.

In the present ⁴⁵Ca uptake experiments, different results were obtained between the muscles incubated in *hyper*-40 K and *iso*-152 K media using the same experimental planning

FIG. 2. Changes in ⁴⁵Ca uptake and tissue Ca of guinea pig taenia coli in *hyper*-40 K and *iso*-152 K media.

● : Normal medium (2.7 mM K, 149.1 mM Na)
 ○ : *Hyper*-40 K medium (42.7 mM K, 149.1 mM Na)
 □ : *Iso*-152 K medium (151.8 mM K, 0 mM Na)

Each point is an average of 6 to 13 determinations. S.E. indicated where possible.

as Urakawa and Holland (1), suggesting that the difference could not have resulted from 4 minute-wash. Further, as shown in the results, Ca content of taenia coli increased in the medium containing higher concentration (2.5 mM) of Ca than the cells (2.2 mM/kg wet weight) when incubated in *hyper*-40 K medium, which suggests that the increase in total Ca content could not be caused by a squeezing out of extracellular fluid.

Increase in ^{45}Ca uptake and tissue Ca in hypertonic 40 mM K medium in taenia coli have already been reported (1) and Ca outflow from the cells have also been reported not to increase in hypertonic 40 mM K medium (7). From these data, tonic response induced in hypertonic 40 mM K medium is suggested to be maintained by transmembrane Ca transport (1, 8).

On the other hand, there are some reports about the effect of high-K/low-Na medium on the smooth muscle. Imai and Takeda (9) reported that tonic response induced by high-K in low or no Na medium in taenia coli partly depends on the extracellular Ca and the rest of the tonic response draws on a store of "bound" Ca for its evolution. In rat uterine smooth muscle, Van Breeman and Daniel (6) reported the decrease in ^{45}Ca uptake in high-K/low-Na medium and suggested that myometrial contraction is induced by a release of Ca from the inside of the cell membrane.

In *iso*-152 K medium ^{45}Ca uptake of taenia coli did not increase above control value but total Ca content decreased markedly. If it is presumed that the loss of Ca is indispensable in the small tonic response of smooth muscle in isotonic high-K/Na-deficient medium, then it would seem plausible to assume that the conversion of a certain fraction of bound Ca to the free form within the cell is the source of Ca for this response as suggested by both Imai and Takeda (9) and Van Breeman and Daniel (6), while the large tonic tension induce in *hyper*-40 K medium might be maintained by the influx of extracellular Ca as suggested by Urakawa and Holland (1).

The cause of the difference in ^{45}Ca uptake and tissue Ca in *hyper*-40 K and *iso*-152 K media might probably be the different composition in the external medium. *Hyper*-40 K medium contained relatively high K concentration of 42.7 mM and normal Na concentration of 149.1 mM and *iso*-152 K medium contained very high K of 151.8 mM and 0 mM Na. However, from the present data, there is no evidence to clarify which of Na and/or K is the cause of the difference.

SUMMARY

^{45}Ca uptake and tissue Ca of guinea pig taenia coli during K-induced contracture were examined. In *hyper*-40 K medium (42.7 mM K, 149.1 mM Na) large tonic tension averaging 12 g was induced in the muscle and both ^{45}Ca uptake and tissue Ca of the muscle increased, while in *iso*-152 K medium (151.8 mM K, 0 mM Na) only a small tension about 2 g remained and ^{45}Ca uptake did not increase and tissue Ca decreased.

A possible mechanism and cause of this difference are discussed.

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