"CALCIUM-INDUCED RELEASE OF CALCIUM" IN RECTAL SMOOTH MUSCLE OF MICE

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Abstract—Contractile responses to K, ACh, Ba and exogenous Ca of rectal strips from mice were recorded isotonically. These responses consisted of phasic contraction and subsequent tonic contraction. In Ca-depleted preparations exposed to Ca-free bath medium, the contractions by K and ACh disappeared but the contraction by Ba remained constant. This residual contraction by Ba is presumably due to a direct stimulation of Ba to contractile elements of muscle. Metabolic inhibitors (anoxia and DNP) abolished tonic contraction without affecting phasic contraction by exogenous Ca. In Ca-free bath medium, tonic contraction by exogenous Ca immediately disappeared whereas phasic contraction gradually decreased and about 3 hr later remained constant in parallel with the occurrence of residual contraction by Ba. After pretreatment with the removal of Na from bath medium, which produced Ca-release action, phasic contractions by exogenous Ca in Ca-free bath medium were depressed like those by K, ACh and Ba. These results suggest that the phasic contraction by exogenous Ca is produced mainly by release of Ca and partly by influx of Ca. Thus, it is suggested that the same mechanism of Ca-induced release of Ca from the storage sites as described in skeletal muscle is also operating in rectal smooth muscle of mice.

Using Natori's skinned skeletal muscle preparation, Endo et al. (1) proposed a mechanism of "Ca-induced release of Ca from sarcoplasmic reticulum" in 1970. Operation of this mechanism in skeletal muscle was also mentioned by Ford and Podolsky (2) in the same year. Aizu and Bando (3) have recently suggested the possibility that this mechanism is operative in guinea pig taenia coli. An attempt was made herein to determine whether or not a similar mode of Ca mobilization exists in smooth muscles. Contractile mechanisms of K, acetylcholine (ACh) and Ba were also investigated in relation to the mobilization of Ca.

MATERIALS AND METHODS

Rectal strips were prepared from mice of either sex weighing between 30 and 35 g. A muscle segment 1.5 cm long was suspended in a 30 ml muscle bath containing nutrient solution maintained at 25°C and bubbled with air continuously. Composition of the nutrient solution (Locke's solution) was as follows (mM): NaCl, 154; KCl, 5.6; CaCl₂, 2.2; glucose, 5.6; NaHCO₃, 4.8 (pH 7.2–7.4). Ca-free bath medium was prepared in essentially the same fashion except that CaCl₂ was omitted and osmotic adjustment was not made. The Na-free bath medium was prepared in the same way but NaCl was replaced by LiCl 6.53 g/l for the isotonic adjustment, whereas the Na- and Ca-free bath medium was prepared

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in the same way as the Na-free bath medium except that the CaCl₂ was removed.

Tonus of the strip was isotonically recorded on a smoked paper drum with a magnification of approx. 10 times. Preparations were allowed to equilibrate for 70 to 90 min before initiation of the experiment. Usually, spontaneous motility was not observed in this preparation, although a transient motility appeared infrequently. Drug solutions were added to the bath medium in cumulative concentrations and reversed by washing with nutrient solutions. In order to equalize the concentration of Ca in experiments with normal and Ca-free bath media, the dose of Ca applied to the Ca-free media was increased by 2.2 mM, the concentration contained in the normal media. Concentration of drugs is expressed in the term of g/ml of the salts unless otherwise stated.

The drugs employed were: acetylcholine chloride (Tokyokasei), atropine sulfate (Merck, Germany), barium chloride (Wako chemicals), calcium chloride (Yoneyama), 2,4-dinitrophenol (Wako chemicals), lithium chloride (Yoneyama) and potassium chloride (Yoneyama).

RESULTS

**Shape of contraction induced by contractile agents and exogenous Ca**

In this experiment, 10⁻³ potassium chloride (K), 10⁻⁷ acetylcholine chloride (ACh) and 10⁻³ barium chloride (Ba) were used as the approximate ED₅₀.

In the normal bath medium, the addition of K, ACh and Ba caused a rapidly-developing contraction (phasic contraction) followed by a slow contraction (tonic contraction). In the shape of contraction induced by ACh and Ba, phasic and tonic phases could not actually be distinguished, as shown in Fig. 1. Addition of 2.0 mM CaCl₂ (exogenous Ca) to normal medium initiated a contraction that showed a clear fade between phasic and tonic phases. This contraction increased with the increase of Ca, although a very high concentration of Ca (12 mM) resulted in a relaxation. Pretreatment with atropine 10⁻⁷, which inhibits the contraction by ACh (ED₅₀) completely, produced no apparent alteration in the exogenous Ca-induced contraction.

**Effect of metabolic inhibition (anoxia and dinitrophenol):** In this paper, use of the term “anoxia” refers to discontinuation of the aeration to the bath medium. Pretreatment with anoxia or dinitrophenol (DNP) 5 × 10⁻⁷ for 30 min attenuated tonic contractions without affecting phasic contractions by K, ACh and Ba. Tonic contraction by exogenous Ca was abolished completely, although phasic contraction was not affected. Influence of anoxia

![Fig. 1. Effect of anoxia on the contractile responses to stimulant agents: (A) normal conditions, (B) 30 min pretreatment with anoxia.](image-url)
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on the shape of contraction is shown in Fig. 1.

Effect of the removal of Ca from normal bath medium: When normal medium was exchanged for a Ca-free one, a transient contraction lasting for about 1 min appeared. At about 3 min after this exchange of bath medium, phasic contractions by K, ACh and Ba were reduced by 17.1 ± 2.3% (N=6), 11.2 ± 1.4% (N=5) and 9.3 ± 1.3% (N=6), respectively, while the tonic contractions with these agents were 22.4 ± 2.1% (N=6), 13.6 ± 1.2% (N=5) and 11.2 ± 2.1% (N=6), respectively, as compared with those in normal medium. The difference in the magnitude of reduction in phasic and tonic contractions obtained here was not statistically significant (P=0.05). On the other hand, phasic and tonic contractions by exogenous Ca were not modified by the 3 min exposure of the preparation to Ca-free medium as compared with those in normal medium (Fig. 2).

Time course of decrease in the action of contractile agents and exogenous Ca in Ca-free medium

In a Ca-free medium, the tonus gradually fell and remained stable about 160 min later. Contractile responses to drugs also gradually decreased in the Ca-free medium, and the contractions by K and ACh disappeared about 72 ± 5 min (N=8) and 140 ± 12 min (N=8) later, respectively. Contractions by Ba further decreased in the Ca-free medium, but about 192 ± 11 min (N=8) later remained constant at the magnitude of 30–35% as compared with that in normal medium, that is, “residual contraction by Ba” was obtained. Time required for the disappearance of K-induced or ACh-induced contraction in a Ca-free medium was markedly shortened by the prior treatment with Ba-induced contraction.

In preparations which were incubated in a Ca-free medium for about 10 min or longer, shape of the contraction by exogenous Ca changed to exponential in contrast with that in normal medium as shown in Fig. 2. This exponential contraction by exogenous Ca gradually decreased in Ca-free medium and about 3 hr later, when the residual contraction by Ba appeared, this contraction remained at the constant magnitude, that is, “residual contraction by exogenous Ca” was obtained. The exponential contraction including residual contraction by exogenous Ca was not affected by metabolic inhibitors (anoxia and DNP) (Fig. 2).

Influence of Ca removal from normal medium on the concentration-action curves of the phasic contraction by exogenous Ca

For exclusion of the tonic component of the contraction by exogenous Ca, the experi-
ments were carried out under anoxia. Concentration-action curves were prepared by the cumulative dose method. Exogenous Ca was added to the bath medium in three different conditions: (A) in normal medium, (B) in Ca-free medium when the contraction by K disappeared and (C) in Ca-free medium as the residual contraction by Ba occurred. As shown in Fig. 3, the log. concentration-action curve for exogenous Ca of (B) showed the shift of a marked downward compression and that of (C) still more, as compared with that of (A).

Fig. 3. Influence of Ca-removal from the normal medium on the concentration-action curves for exogenous Ca. All curves were prepared by the cumulative dose method under anoxia. (A) in normal medium, (B) and (C) in Ca-free medium: (B) when K(ED50)-induced contraction disappeared and (C) when residual contraction by Ba(ED50) occurred.

Effect of the removal of Na from normal medium

When NaCl was removed from normal medium and substituted by equiosmotic LiCl, a rise in tonus appeared and returned to the initial tonus within 3 min. This contraction was not modified by either the pretreatment with atropine 10⁻⁷ or with anoxia for 30 min. In a Ca-free medium, this transient contraction was reduced to 80.3±4.2% (N=7) 3 min later and then gradually decreased and finally disappeared in parallel with the loss of K-induced contractions.

As shown in Fig. 4, pretreatment with Na removal for 3 min before the exchange of bath medium to Ca-free from normal depressed the phasic contractions in Ca-free medium by K, ACh, Ba and exogenous Ca by 40.4±1.4% (N=6), 26.3±2.1% (N=6), 15.4±1.3% (N=6) and 20.3±2.2% (N=8), respectively, as compared with those obtained without this pretreatment. The same pretreatment attenuated tonic contractions by K, ACh and Ba, but increased tonic contraction by exogenous Ca in Ca-free medium.

DISCUSSION

In excised mouse rectum that was immersed in normal medium, the application of K,
ACh and Ba caused an initial fast contraction (phasic contraction) followed by subsequent slow contraction (tonic contraction). Tonic contractions by these drugs were attenuated by the pretreatment with metabolic inhibitors (anoxia or DNP), while phasic contractions were still unaffected. Three min after Ca was removed from the normal medium, the phasic and tonic contractions by K, ACh and Ba were reduced slightly. Thus, it can be considered that in the contractile responses to K, ACh and Ba, phasic contractions are caused mostly by the release of Ca and partly through the passive influx of Ca, whereas tonic contractions are maintained by the active transportation of Ca in which presumably active influx of the Ca released during the phasic contractions may be included.

This study demonstrated that contractions induced by K, ACh and Ba gradually decreased in preparations exposed to Ca-free medium, and K-induced and ACh-induced contractions disappeared 72 ± 5 min and 140 ± 12 min later, respectively, whereas the contractile response to Ba further decreased in Ca-free medium and 192 ± 11 min later it remained constant, that is, “residual contraction by Ba” was obtained. Furthermore, time required for the loss of contractions by K and ACh in Ca-free medium was markedly shortened by the prior treatment with Ba. These findings support the hypothesis that Ca released by K or ACh may be from the loosely bound storage sites, as Hurwitz et al. (4) mentioned, and the Ca-release action of Ba may be concerned with the loosely bound storage sites and also with the tightly bound storage sites of Ca. The result of residual contraction by Ba was consistent with the suggestion of Daniel (5) that contraction by Ba was mediated by the release of Ca as well as via the direct stimulation of Ba to smooth muscle contractile elements.

In normal medium, addition of Ca also initiated a contractile response that contained initial rapidly-developing phasic phase followed by subsequent slow tonic phase and with a clear fade between them. Since this contraction was not influenced by the treatment with atropine, a cholinergic mechanism can be ruled out. In preparations which were treated with metabolic inhibitors (anoxia or DNP), tonic contraction by exogenous Ca was abolished whereas phasic contraction remained unaffected. Three min after the removal of Ca from the normal medium, contractile response to exogenous Ca could be obtained and was exactly the same shape and magnitude as those observed in normal medium when the concentration of Ca was the same. About 10 min or more later, shape of the exogenous Ca-induced contraction turned exponential in the Ca-free medium and was not modified by the treatment with metabolic inhibitors (Fig. 2). Thus, it may be considered that this exponential contraction by exogenous Ca derives from the phasic component and does not include the active transportation of Ca that is responsible for the tonic component. It was also demonstrated that exponential contraction by exogenous Ca gradually decreased with the lapse of time in a Ca-free medium, and became constant in parallel with the occurrence of the residual contraction by Ba, that is, the “residual contraction by exogenous Ca” was obtained. From these results, it is suggested that the phasic contraction by exogenous Ca is caused for the greater part by the release of Ca and in part through the direct passive influx of Ca, the latter being responsible for the residual contraction by exogenous
Release of Ca with the phasic contraction by exogenous Ca was further supported by the analysis with log. concentration-action curve of exogenous Ca concerning phasic component under anoxia, since the curve showed a marked downward compression with the lapse of time after the preparation was exposed to Ca-free medium (Fig. 3).

Removal of Na from normal medium elicited a transient contraction which returned to a normal tonus within 3 min. This transient contraction is not mediated through cholinergic action, since it was not affected by atropine. In the Ca-free medium, transient contraction by removal of Na also appeared, but decreased gradually by repeated treatment and disappeared when the K-induced contraction was lost completely. Thus, this transient contraction probably depends on the release of Ca from the storage sites which are sensitive to K. This mechanism has also been mentioned by Taniyama (6) in rat ileum.

By pretreatment with removal of Na for 3 min before the exchange of bath medium to Ca-free from normal, phasic contractions in Ca-free medium by K, ACh, Ba as well as exogenous Ca were depressed, as compared with those obtained without this pretreatment (Fig. 4). This result may be explained by the assumption that Ca in storage sites was depleted by removal of Na and so the following phasic contractions (depending on release of Ca) by these agents were reduced. On the other hand, tonic contraction by exogenous Ca was potentiated by removal of Na whereas the tonic contractions by K, ACh and Ba were attenuated. This potentiation may be attributed to the fact that the membrane stability was decreased by the depletion of Ca in storage sites and therefore the influx of exogenous Ca was accelerated. Summarizing these findings, it appears that the release of Ca is concerned with the phasic contraction but not with the tonic contraction by exogenous Ca. Tonic contraction by exogenous Ca is probably due to influx of Ca, which is energy-dependent as suggested by the selective depression by metabolic inhibitors as stated above.

From the sum of evidence obtained in the present study, it is suggested that the phasic contraction by exogenous Ca is mainly dependent on release of Ca from the storage sites. Therefore, it is reasonable to assume that the mechanism of Ca-induced release of Ca as described in skeletal muscle (1, 2) could be the same mechanism which operates in the rectal smooth muscle of mice.

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