RELAXING ACTIVITY OF RELAXING FACTORS IN VARIOUS KCl CONCENTRATIONS

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Recently, BRIGGS and KING (1962) and TAKAUJI et al. (1963) have demonstrated that, at relatively low KCl concentration (30 mM or 70 mM), the relaxation of single glycerol-extracted muscle fibers is only slight in the reaction mixture pretreated with a bivalent cation chelating resin, Chelex-100 or Dowex A-1, or in the reaction mixture to which the resin was directly added. On the other hand, SEIDEL and GERGELY (1963) have reported that, at relatively high KCl concentration (130 mM), the development of tension of the single fibers is strongly inhibited by the reaction mixture containing calcium-free ATP pretreated with a resin, Dowex 50.

In the present paper, in order to determine if the discrepancy between these results is explained by the difference of KCl concentration in the reaction mixture used by these authors, the effect of a resin, Dowex A-1, on the glycerol-extracted muscle fibers was studied over the wide range of KCl concentration. Furthermore, the effect of EDTA and microsomes on the fibers was studied in various KCl concentrations.

METHODS

Glycerol-extracted muscle fibers and microsomes were prepared from rabbit skeletal muscle as reported elsewhere (NAGAI et al., 1960; NAGAI and UCHIDA, 1960).

The measurement of tension, the purification of Dowex A-1 and the pretreatment of reaction mixture with the resin were performed as described in the preceding paper (TAKAUJI et al., 1963). It was demonstrated by flame photometry that KCl concentration of reaction mixture was not affected by the pretreatment of the reaction mixture with Dowex A-1.

Calcium depriving activity of Dowex A-1 was determined by using of $^{45}\text{CaCl}_2$ (14 μC of ^{45}Ca /mmole of Ca) as described in the previous paper (TAKAUJI et al., 1963). It was obvious from the determination that 0.02 mM $^{45}\text{CaCl}_2$ added to the reaction

The following abbreviations were used: ATP, adenosinetriphosphate; EDTA, ethylenediaminetetraacetic acid; Tris buffer, tris (hydroxymethyl) aminomethane-acetate buffer.

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mixture containing 70 mM KCl was completely removed by passage of reaction mixture through a Dowex A-1 column.

The laboratory wares were cautiously washed with Dowex A-1 treated solution before use to avoid contamination by extraneous calcium as much as possible.

The concentration of protein was determined by the micro-KJELDAHL method.

Dowex A-1 was obtained from Muromachi Kagaku Kogyo Kaisha, Ltd., (The Dow Chemical Company). ATP was a Sigma crystalline preparation (Sigma 113B-7020). $^{45}\text{CaCl}_2$ was purchased from Japan Radioisotope Association. EDTA was obtained from Dojin Yakugaku Kenkyusho, (Kumamoto), Japan. Other reagents used were commercial products of the best reagent grade available.

RESULTS

In FIG. 1, the time course of the relaxation of glycerol-extracted muscle fibers induced by various relaxing factors at 10 mM KCl and 140 mM KCl

FIG. 1. The time course of the relaxation of glycerol-extracted muscle fibers by various relaxing factors at 10 mM KCl and at 140 mM KCl concentration. Ordinate; the maximum tension of fibers developed in the control reaction mixture containing 66.7 mM Tris buffer (pH 7.0), 2 mM ATP, 2 mM MgCl_2 , and 10 mM KCl was taken as 100%. First arrow ($\downarrow$) indicates when the reaction mixture containing 2 mM oxalate was replaced with the reaction mixture pretreated with Dowex A-1, or with that containing Dowex A-1, EDTA or microsomes, and second arrow ($\downarrow$) indicates the addition of CaCl_2 to these reaction mixtures. Extraction period of fibers, 9 months. Average of maximum tension, 2270 g/cm². Temp., 18~24°C. ○, reaction mixture without any of relaxing factors (10 mM KCl); ●, reaction mixture without any of relaxing factors (140 mM KCl); ⊗, reaction mixture containing 2 mM oxalate; ⊗, 2 mM oxalate-containing reaction mixture pretreated with Dowex A-1; ●, 3 ml of reaction mixture (with 2 mM oxalate) containing 3 ml of Dowex A-1; □, 0.05 mM EDTA; △, 0.05 mg of microsomes/ml. CaCl_2 , 0.02 mM. The solid line represents the reaction mixture containing 10 mM KCl, and the dashed line does that containing 140 mM KCl.

concentrations is indicated. Furthermore, in FIG. 2, the relation between the relaxation and the KCl concentration (10 mM to 210 mM KCl) is demonstrated in detail.

FIG. 2. Relationship between the relaxation of fibers induced by various relaxing factors and KCl concentrations. Ordinate was similarly represented to that done in FIG. 1. The fibers were relaxed by replacement of the control reaction mixture (see legend of FIG. 1) with another that as indicated. In each of experimental series, KCl concentration was over range 10 mM to 210 mM. ○: curve a, reaction mixture without relaxing factors; ⊙: curve b, reaction mixture containing 2 mM oxalate; ⊗: curve c, 2 mM oxalate-containing reaction mixture pretreated with Dowex A-1; ●: curve d, 3 ml of Dowex A-1 was added to 3 ml of oxalate-containing reaction mixture; □: curve e, 2 mM oxalate plus 0.05 mM EDTA; ▲: curve f, 2 mM oxalate plus 0.05 mg of microsomes/ml. Each point indicated in figure is average of 7~10 experiments. Conditions otherwise were the same as stated for FIG. 1.

As shown in FIG. 1 and FIG. 2 (curve c and d), at 10 mM KCl to 140 mM KCl concentrations, the Dowex A-1-pretreated reaction mixture containing 2 mM ATP, 2 mM $MgCl_2$, 2 mM oxalate and 66.7 mM Tris buffer decreased only slightly the tension of fibers (3~6%). Even by the direct addition of Dowex A-1 to the non-pretreated reaction mixture, the relaxation was at most 5~9%. This result is in agreement with that of BRIGGS and KING (1962) at 30 mM KCl and that of TAKAUJI et al. (1963) at 70 mM KCl, respectively, while it is in contrast with that of SEIDEL and GERGELY (1963) at 130 mM KCl.

In the preceding paper, TAKAUJI et al. (1963) reported that at 70 mM KCl concentration, the relaxing activity of microsomes or EDTA was remarkably high under the condition in which the effect of Dowex A-1 was only slight.

In the present experiment (FIG. 1 and FIG. 2, curve e and f), a similar result was obtained over range of 10 mM KCl to 210 mM KCl concentration. In addition, the relaxing activity of 0.05 mM EDTA was scarcely dependent on the KCl concentration (FIG. 1 and FIG. 2, curve e), whereas the relaxing activity of 0.05 mg of microsomes per ml was remarkable at low KCl concentration, especially at about 70 mM KCl and declined at higher KCl concentration.

The fibers relaxed by these various relaxing factors were remarkably re-contracted by addition of 0.02 mM CaCl_2 (FIG. 1).

DISCUSSION

Recently, TAKAUJI et al. (1963) reported that the relaxing activity of microsomes is not explained only by their calcium binding activities, from the result that EDTA or microsomes causes remarkably the fibers to relax, whereas Dowex A-1 which has a strong calcium depriving activity causes only slightly the fibers to relax. Our present results obtained over the wide range of KCl concentration further supported this conclusion.

Furthermore, the result demonstrating that the relaxing activity of microsomes was higher at lower KCl concentration, while that of Dowex A-1 or EDTA was scarcely dependent on KCl concentration (FIG. 2, curve c~f) suggests that the production or the release of relaxing substance by microsomes may be dependent on KCl concentration.

In the experiments on the relaxing activity of a bivalent cation-chelating resin, the reaction mixture used by BRIGGS and KING (1962) and TAKAUJI et al. (1963) contains oxalate and its KCl concentration is relatively low (30 mM or 70 mM), while that used by SEIDEL and GERGELY (1963) does not contain oxalate and its KCl concentration is relatively high (130 mM). However, it appears from FIG. 1 (curve a~d) that the discrepancy between these two results is not due to the difference of these experimental conditions, that is, the relaxing activity of Dowex A-1 was only slight independently on KCl concentration and was at most several per cent at 130 mM KCl concentration in contrast with the result of SEIDEL and GERGELY (1963). Furthermore, the sensitivity of fibers to calcium in the presence of oxalate was similar to that in the absence of oxalate (TANIGUCHI, in press). In the experiment of SEIDEL and GERGELY (1963), the effect of reaction mixture containing the calcium-free ATP treated with Dowex 50 was studied on single muscle fibers relaxed by 1 mM EGTA. Therefore, the possibility could not be excluded that the remarkable relaxing activity of Dowex 50 demonstrated in their results may be ascribed to some influence given on the fibers by EGTA having been remained in the fiber.

SUMMARY

1. The relation between the KCl concentration (10 mM to 210 mM KCl) and the relaxation of glycerol-extracted muscle fibers induced by various relaxing factors was demonstrated.
2. At each KCl concentration, the relaxation of fibers varied at most 3~6% in the Dowex A-1-pretreated reaction mixture and it was also 5~9% even in the reaction mixture to which a sufficient amount of Dowex A-1 was directly added.
3. 0.05 mM EDTA caused markedly the fibers to relax independently on the KCl concentration.
4. The relaxation of fibers induced by 0.05 mg of microsomes per ml was remarkable at relatively low KCl concentration, especially at 70 mM KCl.

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