ACTIONS OF Ba AND Sr IONS ON ISOLATED RAT ILEUM

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It is generally accepted that Ca ion is an essential substance in the contractile response of smooth muscle. There are several reports that action of the Sr ion resembles that of the Ca ion on smooth muscle. Such include the stabilizing action on cell membrane (1, 2) and the direct stimulation to muscle contractile elements to elicit contraction (1, 3, 4). On the other hand, it has been reported that the Ba-induced contraction is dependent on the mobilization of Ca (5-7), that Ba activates contractile proteins (8) or that Ba produces a contraction by these two actions (9). In an attempt to elucidate the discrepancies, actions of Ba and Sr ions were investigated in relation to mobilization of the Ca ion.

Male rats (200-250 g) were sacrificed by a blow on the neck, the ileum was removed and dissected, and strips approx. 1.5 cm long, were fixed vertically with a load of 0.5 g in the preparation bath containing 30 ml of nutrient solution (Locke's solution: NaCl 154, KCl 5.6, CaCl₂ 2.2, glucose 5.6, NaHCO₃ 4.8 (mM)). The medium was maintained at $27 \pm 1^\circ\text{C}$ and continuously bubbled with a mixture of 95% O₂ and 5% CO₂. Muscle contractions were isotonicallly recorded on a smoked paper with an approx. 10X magnification. Preparations were allowed to equilibrate for 90 min in normal media, before experiments were begun. Ca-free solution was prepared in the same fashion as Locke's solution except that CaCl₂ was omitted and 0.01 mM GEDTA was added. K-depolarizing Ca-free solution was prepared in the same way as the Ca-free solution except that 56 mM Na was replaced by an equiamount of K.

Ba (0.5-10 mM) produced a sustained contraction in normal medium. The contraction by Ba (0.5-10 mM) was not modified after a 3 min exposure to Ca-free medium, whereas it gradually decreased by repeated treatments with Ba at an interval of 30 min in Ca-free medium and it then remained at a constant magnitude after approx. 300 min exposure to Ca-free medium, when the K-induced contraction had disappeared. On the other hand, Sr (2-10 mM) produced a sustained relaxation in normal medium, whereas after a 3 min exposure to Ca-free medium, Sr (0.5-10 mM) caused a sustained contraction. The contraction by Sr (0.5-10 mM), like that by Ba, was gradually decreased by repeated treatments with Sr at an interval of 30 min in Ca-free medium and remained at a constant magnitude approx. 300 min after Ca removal. These contractions by Ba and Sr were not modified by 5×10^{-4} mM atropine, which abolished acetylcholine-induced maximal contraction. Thus, the contractions by Ba and Sr were the result of a release of Ca from storage sites, until a constant magnitude was attained in the Ca-free medium. In the following experiments,

FIG. 1. Effect of Ba (a) and Sr (b) on the concentration-action curves of Ca obtained in K-depolarizing Ca-free medium. Cumulative concentration-action curves of Ca were prepared after contractions by Ba and Sr had remained at a constant magnitude in Ca-free medium. Magnitude of maximal contraction by Ca was taken as 100%. ●—●, control; ●—●, in the presence of 1 mM Ba or Sr; ○—○, in the presence of 5 mM Ba or Sr, x—x, concentration-action curve of Ba or Sr. Vertical bars represent S.E. No. of preparations, 8–18.

preparations in which the contractions by Ba and Sr had remained at a constant magnitude in Ca-free media, were exposed to K-depolarizing Ca-free medium. The addition of Ca (0.2–10 mM), Ba (0.5–10 mM) or Sr (0.5–10 mM) to the K-depolarizing Ca-free medium produced sustained contractions. Cumulative concentration-action (C-A) curves of Ca were obtained in K-depolarizing Ca-free medium. As shown in Fig. 1-(a), the log C-A curves of Ca shifted to the left by pretreatments for 10 min with 1 mM and 5 mM Ba, respectively. On the other hand, as shown in Fig. 1-(b), the log C-A curves of Ca shifted to the left in low concentrations of Ca and to the right in high concentrations of Ca by pretreatments for 10 min with 1 mM and 5 mM Sr, respectively (Fig. 1). These shifts of the C-A curves of Ca by Ba and Sr are similar to the competitive interaction of the occupancy theory in drug-receptor interaction (10, 11). Since the log C-A curves of Ca in the absence of Sr and in the presence of 1 mM and 5 mM Sr crossed each other at the point located at the magnitude of maximal contraction by Sr, the effects of 1 mM and 5 mM Sr on the C-A curve of Ca may correspond to the theory of competitive dualism (10) between full agonist, Ca, and partial agonist, Sr. If the full agonist, Ca, and the partial agonist, Sr, are assumed to act on the same receptor site, our findings may be interpreted as follows: the intrinsic activities and the association constants with receptors of Ca and Sr are represented by α , β , K_{Ca} and K_{Sr} , respectively, where $\alpha > \beta$, and the total number of receptors is expressed by R_t . The activity of Ca, Y_{Ca} , is formulated by

$$Y_{Ca} = \alpha R_t \frac{K_{Ca}[Ca]}{1 + K_{Ca}[Ca]} \quad \dots\dots\dots(1)$$

where $[Ca]$ is the concentration of Ca. The dualistic activity of Ca and Sr, $Y_{Ca, Sr}$, is formulated by

$$Y_{Ca, Sr} = R_t \frac{\alpha K_{Ca}[Ca] + \beta K_{Sr}[Sr]}{1 + K_{Ca}[Ca] + K_{Sr}[Sr]} \quad \dots\dots\dots(2)$$

where $[Sr]$ is the concentration of Sr. From the formulae (1) and (2), the pair of coordinates of the crossing point between log C-A curve of Y_{Ca} and that of $Y_{Ca,Sr}$ is $(\frac{\beta}{(\alpha - \beta) K_{Ca}}, \beta R)$. That is, the crossing point is shown to be located at the height of maximal contraction (βR) induced by Sr. Thus, Sr shows competitive dualism to Ca-induced contractions by operating on Ca-receptor, contractile proteins. However, it is possible that Ca ion is, to some extent, present in the tissue in which Sr-induced contraction remained at a constant magnitude in the Ca-free medium. Such has already been suggested to be the case in guinea pig taenia coli (12), therefore, tissue Ca may participate in the contraction by Sr in the rat ileum. On the other hand, the actions of 1 mM and 5 mM Ba on the log C-A curve of Ca may correspond to the theory of competitive synergism (10) between full agonist, Ca, and partial agonist, Ba. Ebashi (1) reported that the sensitivity of myosin B to Ba ion of barnyard fowl gizzard was lower than that to Sr ion. Furthermore, the affinity of Ba for the contractile protein is lower than that of Sr in glycerinated muscle of guinea pig taenia coli (13). Since the affinity of Ba for the Ca-receptor, contractile proteins, may also be low in rat ileum, Ba would show a competitive synergism to Ca-induced contraction, not a competitive dualism. From these findings, it appears that Ba and Sr ions induce a release of Ca from storage sites to elicit contractions. In the absence of external Ca, these ions would replace Ca in generating contraction by binding to contractile proteins. Furthermore, intracellular Sr ion would antagonize competitively the binding of Ca ion to contractile proteins.

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