Short communication

Effects of Na^+ on the Process of Filling and Depletion of Carbachol Released Ca^{2+} Store in the Guinea-Pig Taenia Coli Smooth Muscle

Th. V. BURDYGA[1] and I. S. MAGURA[2]

1 *Department of General Physiology, Institute of Physiology, Kiev State Shevchenko University, Kiev 17, 252017, USSR*
2 *Department of General Physiology of the Nervous System, Bogomoletz Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev 24, 252601 GSP, USSR*

Previously it has been found that carbachol can cause strong transient contractures of taenia coli smooth muscle in Ca^{2+}-free EGTA-containing solution. This effect was thought to reflect the release of Ca^{2+} from an intracellular store (Brading and Sneddon 1980). The store could be refilled by a brief incubation of the tissue in Ca^{2+}-containing solution and this process was found to require intracellular Na^+ (Brading et al. 1980). Recently it has been found that external Na^+ stimulates Ca^{2+} depletion of the carbachol released Ca store in taenia smooth muscle (Hisayama and Takayanagi 1985). It has been proposed by Brading (1976) that filling of the store from the outside could be via an ion exchange mechanism. Na-Ca exchange is likely to mediate this process.

The present study was undertaken to investigate the effects of Na ions on both filling and depletion of carbachol released Ca^{2+}-store in guinea-pig taenia coli smooth muscle.

The experiments were performed on isolated pieces of taenia coli from guinea-pig using the superfusion technique for tension recording (Brading and Sneddon 1980).

The composition of normal Krebs solution was (in mmol/l): Na^+, 136.9; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 133.5; $H_2PO_4^-$, 1.2; HCO_3^-, 15.5; glucose, 11.5 bubbled with 97 % O_2 + 3 % CO_2 at 36—37 °C. Loading of the tissue with Na ions obtained by inhibiting the Na-K pump by treating the taenia with ouabain form 60 min as described rearlier (Aickin et al. 1984). Ca^{2+}-free solutions were prepared by replacing all $CaCl_2$ by $MgCl_2$ and 3 mmol/l EGTA was added. Control response to 10 s applications of 10^{-4} mol/l carbachol in normal Krebs solution was established at the beginning of each experiment in normal Krebs and subsequent responses were expressed as percentages of this control.

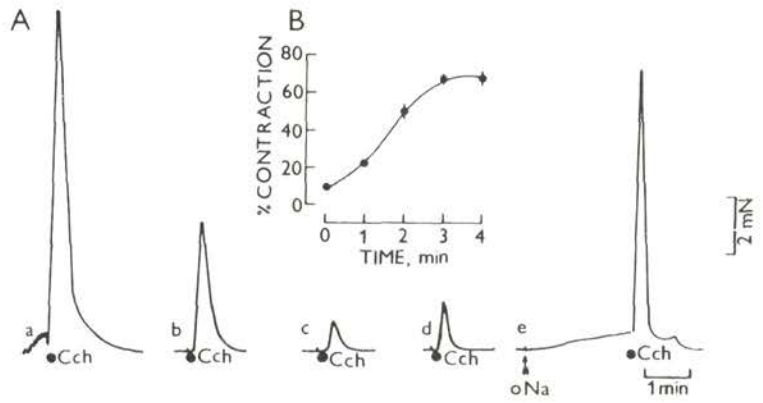

Fig. 1. *A*. Effect of nifedipine (10^{-5} mol/l) on the contractile response of untreated taenia coli to a 20 s application of carbachol (10^{-4} mol/l):a, control response in Krebs solution obtained in the absence of nifedipine; b, c, reduction of the carbachol contracture following 3 and 10 min exposure of the tissue to nifedipine (10^{-5} mol/l); d, partial recovery of the carbachol response following 60 min pretreatment of the tissue with ouabain (10^{-4} mol/l); e, tonic tension and carbachol response of the Na^+-loaded taenia coli caused by respective withdrawal of Na_o^+ and application of carbachol (10^{-4} mol/l) in Na^+-free solution. *B*. Curve to illustrate the time dependence of the store filling on the time of preincubation of the Na^+-loaded taenia in Na^+-free solution. The size of the store is measured as the contractile response to 10^{-4} mol/l carbachol (20 s) expressed as percentage of the control response of the tissue to a 20 s application of 10^{-4} mol/l carbachol in normal Krebs. Mean values are shown; vertical bars indicate SE of the mean ($n = 5$).

Preliminary electrophysiological experiments showed that nifedipine (10^{-5} mol/l) completely blocked both spontaneous and evoked electrical and mechanical activity of the guinea-pig taenia coli. The stimulatory action of carbachol (10^{-4} mol/l) was progressively supressed by nifedipine (10^{-5} mol/l) (Fig. 1*A*). When the carbachol response was virtually abolished the tissue was exposed to 10^{-4} mol/l ouabain for 60 min in the continuous presence of nifedipine (10^{-5} mol/l). Ouabain-treated taenia coli has been shown to gain Na^+ (Casteels 1966). Fig. 1*A*,e shows that Na^+-loaded taenia coli responded to removal of external Na^+ with slowly rising tonic tension. Under these conditions the carbachol response recovered almost completely (Fig. 1*A*,e). The size of the carbachol contraction was found to depend on the time of preincubation of the Na^+-loaded taenia in Na^+-free solution. Graph in Fig. 1*B* illustrates that the store is saturated after 3 min preincubation of the tissue in Na^+-free solution.

Previously it was found that in Ca^{2+}-free solution the store of untreated taenia coli relatively quickly lost its Ca content (Brading and Sneddon 1980).

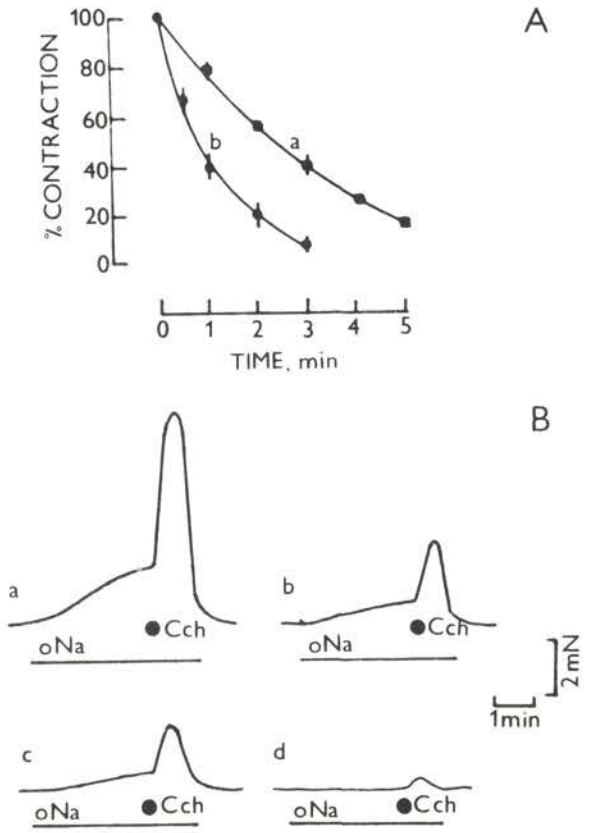

Fig. 2. *A*. Graph to illustrate the time course of the loss of stored Ca^{2+} in Ca^{2+}-free EGTA (3 mmol/l) solution without (curve a) and with (curve b) Na_o^+. The size of the store was measured as the contractile response ot 10^{-4} mol/l carbachol and expressed as percentage of the control response of the tissue to a 20 s application of carbachol in Na^+-free Ca^{2+}-containing solution. The procedure was repeated at different intervals in random order. Mean values are shown; vertical bars indicate SE of the mean ($n = 5$). *B*. a, Na^+-free contracture and carbachol response in the absence of amiloride; b, c, d, Na^+-free contractures and carbachol responses obtained after 5 min exposure of the tissue to 10^{-4}, 2×10^{-4} and 5×10^{-4} mol/l of amiloride, respectively. Records from the same preparation.

Fig. 2 shows the decline in the size of the carbachol contracture on exposure to Ca^{2+}-free solution. Curve *a* shows the result of a 3 min exposure of the tissue to Na^+-free Ca-containing solution before the application of Ca^{2+}-free Na^+-free solution. The store became depleted in Ca^{2+}-free solution within 5—7 min. When the same procedure was repeated, however in the presence of Na_o^+, the rate of depletion of the Ca^{2+}-store was significantly increased (Fig. 2, curve b).

Amiloride, a putative blocker of Na^+-H^+ and the Na^+-Ca^{2+} exchange in other tissues (Siegel et al. 1984), in a concentration range of 10^{-4}—5×10^{-4}mol/l dose-dependently inhibed both Na^+-free and carbachol contracture of the Na^+-loaded taenia (Fig. 2*B*).

The results obtained suggest that in experimental conditions as described above the size of the carbachol-operated Ca^{2+} store can be controlled by Na-Ca exchange.

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