Short communication

Effects of Sodium Gradient on the Cytosolic Free Ca^{2+} and Contractility of the Pregnant Rat Myometrium Smooth Muscle

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Experiments performed on sarcolemmal vesicles isolated from uterine smooth muscle suggested the existence of two Ca^{2+} transporting systems (ATP-driven Ca^{2+} pump and Na^+/Ca^{2+} exchange) (Kosterin et al. 1984). The functional role of these two energy-dependent Ca^{2+} transporting systems in the regulation of $[Ca^{2+}]_i$ and in the contraction-relaxation cycle of intact smooth muscle has not been identified. A kinetic analysis of the two systems showed that the Na^+-dependent Ca^{2+} transporting system had a lower affinity for Ca^{2+} than the ATP-driven Ca^{2+} pump (*K*m for Ca^{2+} was 30—60 μmol/l and 0.3—0.5 μmol/l respectively) (Kosterin, 1990). These results were obtained in experiments performed on a model system of plasma membrane vesicles in which all physiologically relevant conditions, i. e. ionic gradients and membrane potential, were not strictly observed. Nevertheless, the data obtained suggested that, at least in the uterine smooth muscle Na^+/Ca^{2+} exchange does not play a dominant role in regulating cytosolic free Ca^{2+}. However, direct measurement of cytosolic free Ca^{2+} in relation to sodium gradient was obviously required to support this view.

In present work the effect of Na^+ gradient on the level of intracellular free concentration of Ca^{2+} was studied in cell suspension isolated from pregnant rat myometrium using the Ca^{2+}-sensitive fluorescent probe fura-2 (Grynkiewicz et al. 1985). Also, the role of the Na^+ gradient in the regulation of mechanical activity of intact strips dissected from pregnant rat myometrium was investigated.

Suspension of smooth muscle cells from pregnant rat myometrium was prepared by collagenase digestion as described elsewhere (Mollard et al. 1986). The viability of the isolated cells was assessed by trypan blue and ethidium bromide exclusion using the cytospectrofluorimetric method. According to these measurements at least 60—70 % of the cells excluded the dyes. If not indicated otherwise, the $[Ca^{2+}]_i$ measurement studies were performed in the basal medium containing (mmol/l): NaCl 131.4, KCl 5.9, KH_2PO_4 0.44, Na_2HPO_4 0.26, $MgCl_2$ 1.0, $CaCl_2$ 1.0, glucose 11.5, HEPES 5.0 at pH 7.4. To

load smooth muscle cells with fura-2 the cells suspension was incubated with 2.5 μmol/l fura-2/AM at 37°C for 45—60 min. Hydrolysis of fura-2/AM was monitored by the gradual shift in the excitation spectrum from that of the ester, peaking at 370 nm to that of the final indicator, peaking at 340 nm. Fluorescence of fura-2 loaded cells was measured at 37°C in a Hitachi 650—10S fluorescence spectrophotometer with a thermostatted cell holder, using an excitation wavelength of 340 nm (slit 2 nm) and emission wavelength of 495 nm (slit 10 nm). Calculations of $[Ca^{2+}]_i$ were performed in accordance with the method described in detail elsewhere (Grynkiewicz et al. 1985) using the following equation:

$$[Ca^{2+}]_i = K_d (F - F_{min})/(F_{max} - F)$$

where K_d is the apparent dissociation constant of the fura-2-Ca^{2+}-complex assumed to be 224 nmol/l (Grynkiewicz et al. 1985), F is the experimentally obtained fluorescence value, F_{max} is the maximal fluorescence of the fluorescent probe in the presence of saturating Ca^{2+} and F_{min} is the minimal fluorescence in the presence of minimal Ca^{2+}. To obtain F_{max}, the cells were permeabilized with the detergent Berol 185 (final concentration 0.1 %) in the medium containing 1 mmol/l Ca^{2+}. F_{min} was obtained by the subsequent addition of 0.5 mmol/l Mn^{2+}. Extracellular probe or probe in leaky cells was estimated by adding Mn^{2+} (0.05 mmol/l) to aliquots of suspensions of intact and fura-2 loaded cells. The immediate decrease in fluorescence after the addition of $MnCl_2$ (extracellular probe) was 15—25 % of the total fluorescence. The value of $[Ca^{2+}]_i$ was corrected by subtracting the fluorescence of the extracellular probe. In the present experiments cells were used which yielded basal intracellular Ca^{2+} values of 160—260 nmol/l (Fig. 1*a*).

The superfusion technique as described by Brading and Sneddon (1980) was used for tension recording of intact muscle strips. The modified physiological solution used for the perfusion of intact muscle strips had the following composition (mmol/l): NaCl 120.4, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, glucose 11.5. The solution was buffered with Tris- HCl (16.7 mmol/l) at pH 7.4 and maintained at 37 °C. When extracellular sodium was reduced, NaCl was isotonically replaced by either KCl, choline chloride or Tris as specified in the text.

The experiments showed that replacement of all $[Na^+]_o$ with choline had no detactable effect on the concentration of cytosolic free Ca^{2+} in smooth muscle cells with basal levels of intracellular Na^+ (Fig. 1*b*). Treatment of the cells with either the Na^+, K^+-ATPase inhibitor ouabain (1 mmol/l) for 35—40 min, or with Na^+-ionophore monensin (0.05 mmol/l) for 10 min, both procedures being known to rise intracellular Na^+, produced small elevation of the basal levels of $[Ca^{2+}]_i$ (10—20 %). In contrast, Na^+-loaded cells showed a significant increase

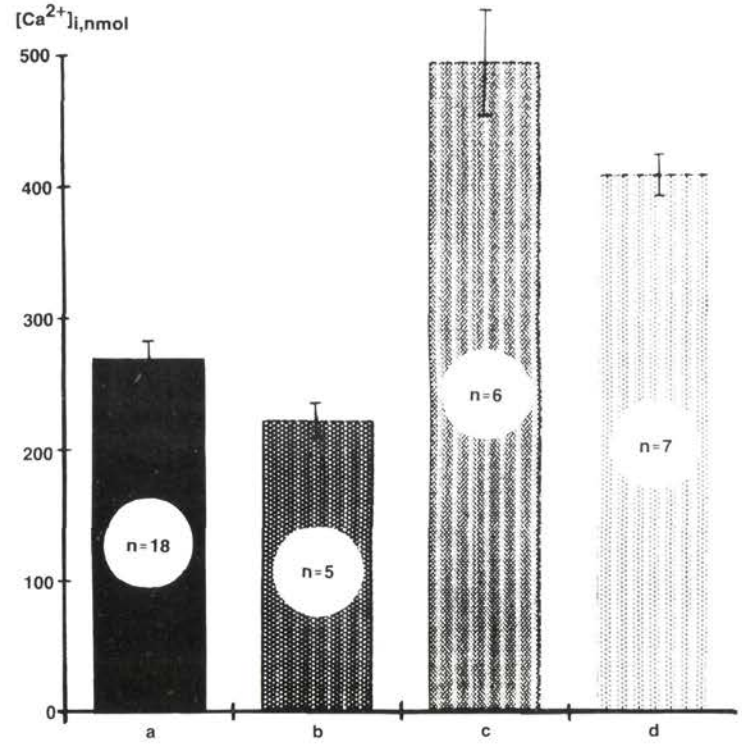

Fig. 1. Effects of sodium gradient manipulation on the level of cytoplasmic free Ca^{2+} in isolated myometrial smooth muscle cells measured with fura-2: control cells (*a*); cells with basal level of intracellular sodium placed in Na^+-free (choline substitution) medium (*b*); cells treated with ouabain (1 mmol/l) for 30 min (*c*) or monensin (0.05 mmol/l) for 10 min (*d*), placed in Na^+-free (choline substitution) medium.

of $[Ca^{2+}]_i$ upon removing $[Na^+]_o$ (Fig. 1*c*, *d*). Data from contraction experiments were in good correlation with the data concerning $[Ca^{2+}]_i$ (Fig. 2). Exposure of the muscle strips to basal levels of Na^+ failed to elicit any contracture in response to removal of $[Na^+]_o$. Also, superfusion of the smooth muscle strips with either ouabain (1 mmol/l) for 30—45 min or monensin (0.05 mmol/l) for 10 min only slightly increased the resting tension (Fig. 2). A strong contractile response did occur, however, when Na^+-loaded tissue was exposed to Na^+-free solution irrespective of the Na^+ substitutes used (Fig. 2). Earlier it was shown that the Na^+-free contracture of the Na^+-rich smooth muscles including pregnant rat myometrium (Aickin et al. 1984; Savineau et al. 1987) reflects the operation of the Na^+/Ca^{2+} exchange in a reverse mode.

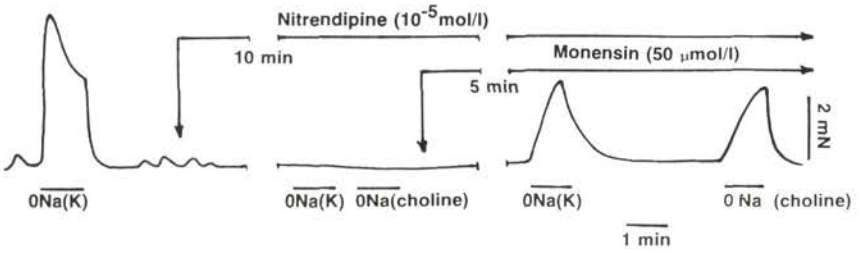

Fig. 2. Effects of monensin (0.05 mmol/l) on the conctractile behaviour of the myometrium upon manipulating the sodium gradient. To exclude any possible involvement of Ca^{2+} channels, the experiments were performed in the continuous presence of nitrendipine (0.01 mmol/l). Note that monensin (0.05 mmol/l) produced a small rise in resting tone, induced recovery of the high-K^+ (126 mmol/l) contracture preliminarily blocked by nitrendipine, and enabled the tissue to contract in response to Na^+ withdrawal.

Our experiments were performed under experimental conditions in which possible intracellular or hormone controlled mechanisms regulating Na^+/Ca^{2+} exchange activity were not considered. However, there are two important points which emerge from this study. First, Na^+/Ca^{2+} exchange does exist in myometrial smooth muscle cells. Second, the fact that untreated smooth muscle cells of pregnant rat myometrium maintain basal levels of $[Ca^{2+}]_i$ and remain relaxed in the complete absence of an inwardly directed sodium gradient makes it clear that Na^+/Ca^{2+} exchange does not play a key role in $[Ca^{2+}]_i$ regulation in resting uterine smooth muscle cells. Therefore, there is substantial ground to presume that it is the high affinity Ca^{2+} pump ($K_m = 0.3—0.5$ μmol/l for Ca^{2+}) of the myometrium cells plasma membrane that plays a key role in maintaining low $[Ca^{2+}]_i$ in resting myocytes. As far as Na^+/Ca^{2+} exchanger is concerned, the possibility remains that having a lower affinity for Ca^{2+} ($K_m = 30—60$ μmol/l) this system is likely to be involved in regulation of $[Ca^{2+}]_i$ during Ca^{2+} overload of the cell.

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