A CALCIUM REVERSAL PHENOMENON: DIFFERENTIATION OF EXCITATORY AND INHIBITORY ROLES OF Ca IN UTERINE SMOOTH MUSCLE CONTRACTION

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Ca ion is known to play dual roles in smooth muscle; an excitatory role in contraction (1-3) and an inhibitory role in membrane stabilization (4). Use of an appropriate blocker should enable differentiation of one role from the other as in the case of adrenoceptors (5). Studies on the differentiation of these two roles of Ca in smooth muscle contraction were done and our findings are reported herein.

Wistar rats, weighing 170-220 g, were ovariectomized. After a recovery period of 5 days or longer, these rats were given estradiol benzoate s.c. (0.1 mg/kg) once daily for 3 days and then sacrificed. The uterine horns were removed and mounted in a 30 ml organ bath containing Locke-Ringer's solution aerated with a mixture of 95% O₂ and 5% CO₂ at 30°C. This solution had the following composition (mM): NaCl 154, KCl 5.63, CaCl₂ 2.16, MgCl₂ 2.10, NaHCO₃ 5.95, glucose 5.55 (normal solution). Ca free solution was of the same composition, except for the omission of CaCl₂. Mechanical responses of the myometrium were recorded on an ink-writing polygraph (RM-45) with a force-displacement transducer, SB-1T in normal solution and SB-1TH in Ca free solution. (SB-1T, SB-1TH and RM-45; Nihon Kohden). SB-1TH is more sensitive than SB-1T, but is not used for recording a tension over 5 g. As oxytocin at 3×10^{-2} unit/ml induced a tension over 5 g in normal solution, SB-1T was used to record mechanical responses in normal solution, and SB-1TH in Ca free solution. The resting tension was 0.5 g in normal solution and 0.2 g in Ca free solution. After an equilibration period of one hour, oxytocin at 3×10^{-2} unit/ml was applied twice for 15 min periods with a one hour interval. The maximal tension developed was used for normalizing mechanical responses, taking this response as 100% (7.6 ± 0.20 g, $n=36$). Thirty min after the second challenge with oxytocin, the medium was replaced by Ca free solution with 3 mM EGTA added to deplete the tissue of Ca. EGTA was adjusted to pH 7.4 with NaOH. (EGTA; glycoletherdiaminetetraacetic acid). The incubation time was 50 min, and the force-displacement transducer was replaced by SB-1TH. The solution was replaced by Ca free solution without EGTA, and after a further incubation for 20 to 30 min, oxytocin (3×10^{-2} unit/ml in Fig. 1A; 10^{-2} unit/ml in Fig. 1B and 1C) was applied. Oxytocin induced a sustained contraction, even after extensive Ca depletion by EGTA (Ca free contraction). The magnitude of Ca free contraction was 0.42 ± 0.024 g or $5.9 \pm 0.37\%$ ($n=12$) at 3×10^{-2} unit/ml oxytocin and was 0.38 ± 0.020 g or $4.9 \pm 0.21\%$ ($n=24$) at 10^{-2} unit/ml oxytocin. The Ca free contraction was not affected by 3×10^{-6} M D-600, a Ca

FIG. 1. Oxytocin-induced Ca free contraction of rat uterine smooth muscle. A; Effect of D-600 and papaverine (Pap.) on Ca free contraction induced by oxytocin (Oxy.). Papaverine was administered cumulatively. B; Effect of exogenously applied CaCl₂ on Ca free contraction. CaCl₂ was administered cumulatively. Note the relaxation by a low concentration of CaCl₂ (3×10^{-5} M). C; Effect of exogenously applied CaCl₂ in the absence (a) and presence (b) of 3×10^{-6} M D-600. The two uterine horns were from the same animal. Concentrations of D-600, papaverine and CaCl₂ are in molar concentration (M), and that of oxytocin in units/ml. Dots in Fig. 1B and a of Fig. 1C show over-scaled recordings. Note the differences in the scales of tension.

antagonist (6), but was relaxed by papaverine (3×10^{-6} M or more, Fig. 1A), indicating that intracellular Ca is utilized during the Ca free contraction (7). Figure 1B and a of Fig. 1C show the effect of exogenous CaCl₂ applied during Ca free contraction. A low concentration of CaCl₂ (3×10^{-5} M) inhibited the Ca free contraction and a possible participation of contaminated Ca in the Ca free contraction was excluded (Fig. 1B). On raising CaCl₂ concentration to 10^{-4} M, only one large phasic contraction was evoked and this was followed by a relatively quiescent period and much smaller contractions. A further increase in CaCl₂ concentration to 3×10^{-4} M evoked phasic and rhythmic contractions (Fig. 1B and a of Fig. 1C). The contractions evoked by exogenously applied CaCl₂, 10^{-4} M or over are attributed to an influx of Ca into the cell as the muscle had been depleted of Ca. The inhibitory effect of low concentrations of Ca (3×10^{-5} M and possibly 10^{-4} M) would then be more manifest if the influx of Ca is blocked by D-600. Fig. 1C shows that such is indeed the case. Without pretreatment with D-600 (a of Fig. 1C), CaCl₂ (10^{-4} M or more) evoked contraction, whereas, with pretreatment with D-600 (b of Fig. 1C), the low concentration of CaCl₂ (10^{-4} M) did not evoke contraction, rather produced a relaxation of the Ca free contraction. Lanthanum, procaine and quinidine also relaxed the oxytocin-excited Ca free contraction (data not shown). Ca and all these agents have a membrane stabilizing action (8–10). Thus, the relaxation could be attributed to the membrane stabilizing action of Ca. This action is

considered to prevail at the CaCl_2 concentration of 3×10^{-5} M, where, even in the absence of D-600, the Ca free contraction was relaxed by CaCl_2 alone (Fig. 1B). The experiments were repeated on three other preparations with consistent results.

The phenomenon in Fig. 1C is similar to "adrenaline reversal" (11), and thus may be termed "Ca reversal". In "adrenaline reversal", the inhibitory β -adrenergic effect is unmasked by α -blockers. In "Ca reversal", D-600 blocks the influx of Ca utilized for muscle contraction, thus unmasking the inhibitory membrane stabilizing action of Ca. In contrast to the role in muscle contraction (1-3), the role in membrane stabilization is poorly understood. Our findings should contribute to a better understanding of the membrane stabilizing action of Ca.

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