Utilization of a Cellular Calcium Pool for Contraction of the Human Myometrium

Mechanical activity of smooth muscle, like that of skeletal muscle, is regulated by changes in the level of free Ca ions in the myoplasm[1,2]. Stimulants and depressants of smooth muscle appear to act by elevating and lowering the myoplasmic Ca concentration. Various proposals have been made with regard to the source of the Ca ions that enter the cytoplasm.

Acetylcholine and other stimulants of the myometrium may elevate myoplasmic Ca by increasing the permeability of membrane to external Ca, by molilizing bound Ca from cellular stores or by a combined effect of the two processes[1,2]. In spite of an early demonstration that contractile responses by drugs could be elicited from depolarized smooth muscles in Ca free solutions and of other data in support of a cellular pool of Ca[3], the existence of an intracellular store of the activator Ca in smooth muscles is not unequivocally accepted[4–6]. The results presented here give strong evidence for the existence of a cellular calcium pool in the human myometrium from which Ca can be mobilized by acetylcholine to induce contraction.

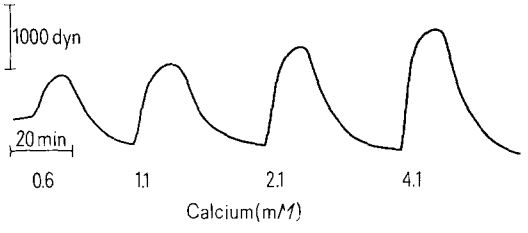

Fig. 1. Contractile response of K-depolarized human myometrium to varying Ca concentrations in Krebs-Ringer medium.

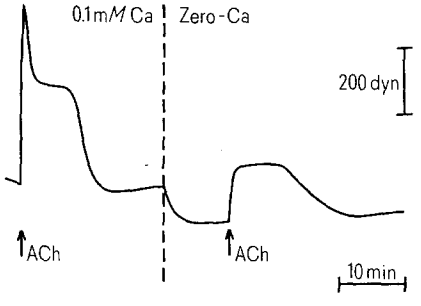

Fig. 2. Response of K-depolarized human myometrium induced by acetylcholine in 0.1 mM (low Ca) and zero-Ca media. Acetylcholine (ACh) concentration was 10^{-4} g/ml.

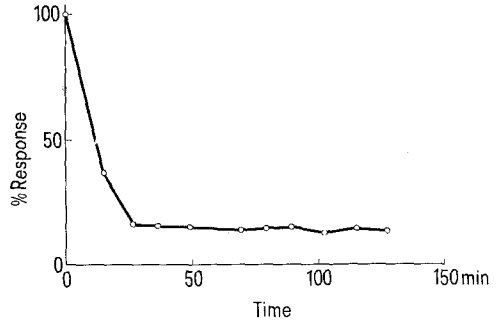

Fig. 3. Repeated contractions of K-depolarized human myometrium in zero-Ca medium. At 0 min the bath solution was changed from low Ca to zero-Ca. Responses were induced by ACh (10^{-4} g/ml) at symbols (○) in the figure.

Myometrial strips (0.5 × 3 × 15 mm) from non-pregnant tissues removed at hysterectomy were mounted in a 20 ml organ bath filled with Krebs-Ringer bicarbonate solution. The composition of this solution was mM: NaCl 115, KCl 4.63, $CaCl_2$, 2.47, $MgSO_4$, 1.16, $NaHCO_3$, 21.9, NaH_2PO_4 1.16, glucose 5.0.

Two other solutions were used in these experiments and are referred to as low Ca where Ca in the Krebs-Ringer solution was reduced to 0.1 mM, and zero-Ca where Ca was omitted but 1 mM EGTA was added. In the depolarizing medium, all of the NaCl was replaced by KCl.

Mechanical responses were recorded isometrically at 37 °C. Illustrations are traced from original recordings, omitting the periods of washings and solution changes.

When K-depolarized myometrial strips were exposed to increasing Ca concentrations (0.6–4.1 mM), graded mechanical responses were obtained (Figure 1). This clearly indicates that external Ca can directly cause contraction of the depolarized myometrium, which is essentially in agreement with the results obtained by EDMAN and SCHILD[3] on depolarized rat uterus. Figure 2 compares the responses of the depolarized myometrium induced by acetylcholine in zero-Ca and Ca-containing solution. The fact that the responses to acetylcholine were considerably faster (Figure 2) than those induced by the addition of Ca (Figure 1) suggests that a cellular pool of Ca is being utilized in the former case. This is further supported by the results in Figure 2 showing that acetylcholine was able to induce contraction in zero-Ca medium (which contained 1 mM EGTA). Changing the medium from low Ca to zero-Ca resulted in 67% decline of resting tension. Significant response to acetylcholine was obtained in zero-Ca medium. This response was considerably reduced when compared with that obtained in low Ca medium (Figure 2). The type of response obtained in zero-Ca medium was also different from that in low Ca medium. In Ca-containing medium, a sharp peak followed by a sustained contracture (a biphasic response) was observed (Figure 2), while in zero-Ca medium the peak was abolished and only the sustained contracture (a monophasic response) was obtained.

EDMAN and SCHILD[3], in their study on the depolarized rat uterus, observed a loss of response in successive contractions induced by acetylcholine in a Ca-free medium. These authors observed that after 1 h, when several repeated contractions by acetylcholine had been induced, the responses became insignificant. A similar loss of response to stimulants of other smooth muscles in Ca-free medium has been reported (e.g.[1,2]). In fact certain mammalian smooth muscles failed to respond to acetylcholine in Ca-free medium[7]. Figure 3 shows that the mechanical response

[1] E. E. DANIEL, *Muscle*, Eds. W. M. PAUL, E. E. DANIEL, C. M. KAY and G. MONCKTON; (Pergamon Press, New York 1965), p. 295.
[2] L. HURWITZ and A. SURIA, A. Rev. Pharmac. *11*, 303 (1971).
[3] K. A. P. EDMAN and H. O. SCHILD, J. Physiol., Lond. *161*, 424 (1962).
[4] A. P. SOMLYO and A. V. SOMLYO, Pharmac. Rev. *20*, 197 (1968).
[5] C. L. SEIDEL and D. F. BOHR, Circulation Res. Suppl. II, *28*, 88 (1971).
[6] E. E. DANIEL, A. Rev. Pharmac. *4*, 189 (1964).
[7] J. M. POTTER and M. P. SPARROW, Aust. J. exp. Biol. Med. Sci. *46*, 435 (1968).

induced by acetylcholine in zero-Ca medium remained unchanged for 2 h, in which time 9 successive contractions were induced. It appears therefore that not only different smooth muscles differ in their Ca binding properties but the same muscle from different species may differ in this respect[8,9]. The present results, showing that there was no decline in response in successive contractions induced by acetylcholine in zero-Ca medium, indicate that Ca which is mobilized by acetylcholine is located either intracellularly or on the inner surface of the cell membrane. This Ca, during its mobilization for contraction, does not appear to leak out into the extracellular medium, since in that event the diffused Ca would be captured by EGTA in the external medium (see methods) and would not be available for the consecutive contractions (Figure 3). Alternatively, the cellular calcium pool is enormously greater than the fraction mobilized during a contraction. No information on the precise location of this Ca or the mechanism of its release and re-accumulation can be given. Preliminary experiments on isolated mitochondria and microsomes from this tissue showed that acetylcholine in the concentration used in the present experiment had no effect on Ca binding or release by these fractions[9,10,11].

Zusammenfassung. Im K-depolarisierten Myometrium ist die mechanische Aktivität durch die extrazelluläre Ca-Konzentration graduierbar. Zugabe von Acetylcholin in die Ca-freie, depolarisierende Lösung ergibt mechanische Spannungsentwicklung des Myometriums.

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[8] L. Hurwitz, P. D. Joiner, S. von Hagen and C. R. Davenport, Am. J. Physiol. *216*, 125 (1969).
[9] S. Batra, Am. J. Obstet. Gynec. *112*, 851 (1972).
[10] S. Batra, Biochim. biophys. Acta *305*, 428 (1973).
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Temperature Responses of Exercizing Dogs to Infusion of Electrolytes

In man, equilibrium levels of rectal temperature (T_{re}) during exercise are highly correlated ($r = +0.71$) with plasma sodium and osmotic concentrations, but are essentially unrelated ($r = +0.34$) to variations in plasma volume[1-3]. This ion-osmotic factor appears to act by controlling sweat gland function; that is, the rate of sweating is inversely proportional to the plasma ionic-osmotic concentration. It is not clear if the ions act directly on the sweat glands or if the action is primarily on the hypothalamus. Hasama[4] was one of the first to observe the relationship between plasma ionic concentration and body temperature in resting animals and more recently Myers and Yaksh[5] found that solutions of 3 to 5 times normal concentration of sodium injected into the cerebral ventricles of monkeys increased resting temperature and similar concentrations of calcium decreased body temperature. They postulated that the setpoint for body temperature during rest was determined by the Na^+/Ca^{++} ratio. In the present study the effect of infusions with solutions of various ionic and osmotic composition on exercise temperature responses was studied in dogs, who do not regulate their temperature by sweating.

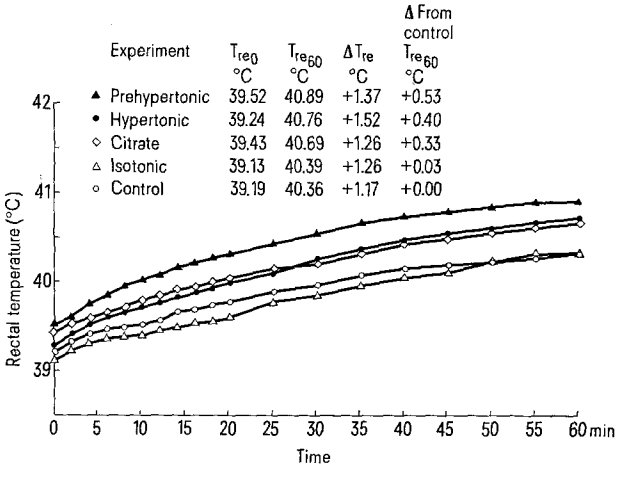

Fig. 1. Average (± S.E.) rectal temperature responses at rest (0-min) and during 60 min of exercise for the 5 experiments.

Material and methods. Six, male, mongrel dogs (11.6 to 27.2 kg) maintained on a standard diet were used. 24 h before each experiment they were deprived of food, but had free access to water. In all experiments the dogs performed 1 h of standard treadmill exercise (1.2 m/sec; 12° slope). Their T_{re} was measured with a thermistor (Electronic) inserted 13 cm. There were 5 different experiments performed on each dog: a) hypertonic: continuous i.v. infusion of NaCl solutions (6.7% to 10.0%, and 110 to 134 ml at a mean rate of 3.6 ml/min (range 2.6 to 4.1) was given for the first 20 min of exercise and 1.1 ml/min (range 0.7 to 1.3) for the final 40 min; the rate was proportional to the size of the estimated extracellular fluid volume designed to raise plasma osmolality to about 320 and 330 mOsm/l; b) isotonic: 0.9% NaCl was infused during the run at the same rate as in (a); c) prehypertonic: the same osmotic load as in (a) was infused during 30-min starting 1 h before exercise; d) control: 1 h of exercise with no infusion; and e) citrate: 3.8% sodium citrate was injected i.v. at a dose of 1.8 ml/kg immediately before exercise.

The infusions were given with a Unipan (Model 304) peristaltic pump. Plasma osmolality (Fiske Osmometer), plasma proteins (Biuret method), plasma sodium (Zeiss flame photometer), and micro-hematocrit (Unipan Model 316) were measured on the 0, 5, 15, 25, 40 and 60 min venous blood samples. The results were analyzed by the *t*-test for paired data with the level of significance ($P \leq 0.05$).

Results and discussion. At the end of one hr of exercise, the highest mean T_{re} was attained following prehyper-

[1] J. E. Greenleaf and B. L. Castle, J. appl. Physiol. *30*, 847 (1971).
[2] J. E. Greenleaf, *The Pharmacology of Thermoregulation* (Karger, Basel 1973), p. 72.
[3] B. Nielsen, G. Hansen, S. O. Jorgensen and E. Nielsen, Int. J. Biometeorol. *15*, 195 (1971).
[4] B. Hasama, Arch. exp. Path. Pharmak. *153*, 291 (1930).
[5] R. D. Myers and T. L. Yaksh, J. Physiol., Lond. *218*, 60 (1971).