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Stimulation of ^{45}Ca efflux from smooth muscle by extracellular Ca^{2+}

SHANES AND BIANCHI[1] first noted that extracellular Ca^{2+} have a stimulating effect on the ^{45}Ca efflux from skeletal muscle. A few years ago FEINSTEIN[2] and VAN BREEMEN, DANIEL AND VAN BREEMEN[3] showed the same effect in uterine smooth muscle, and both groups concluded that the extracellular Ca^{2+} probably act on the cell surfaces to stimulate ^{45}Ca efflux. This conclusion was based on the following observations. This effect was too fast for a preceding penetration of Ca^{2+} into the cells. EDTA, a chelating agent not able to penetrate cell membranes, increased the ^{45}Ca efflux to about the same extent. Local anesthetics inhibited this Ca^{2+}-stimulated ^{45}Ca efflux in uteri but not in the acellular tendon. The first two observations point to surface sites, while the third indicates that these sites are on the surfaces of the cells. Some specificity was also noted in that Sr^{2+} but not Mg^{2+} or Na^{+} could replace Ca^{2+} in stimulating ^{45}Ca efflux. Ba^{2+} was much less effective than Ca^{2+} or Sr^{2+}. Spontaneous contractions occurring after addition of Ca^{2+} were not responsible for the stimulation of ^{45}Ca efflux, since acetylcholine-induced contractions had no such effect[4]. In addition Ba^{2+} caused vigorous contractures, but had only a small effect on ^{45}Ca efflux.

The indications that the cell surfaces were involved led us to investigate the effect of Ca^{2+} concentration on ^{45}Ca efflux. Uterine horns from immature estrogen-pretreated rats were isolated and incubated in Krebs–Ringer bicarbonate solution containing 1.5 mM ^{45}Ca-labelled Ca^{2+} for 2–3 h. All solutions were kept at 37°, pH 7.4, and were bubbled with 95 % O_2, 5 % CO_2. The uteri were then removed, rinsed in non-labelled Krebs–Ringer bicarbonate solution, followed by a washout of the radioactivity into a series of test tubes containing Ca^{2+}-free Krebs–Ringer bicarbonate solution for 65 min. At this time Ca^{2+} was added to the washout solution to bring its concentration to one of the following: 0.05, 0.1, 0.2, 0.5, 1.0, or 2.0 mM. After 40 min of washout in the Krebs–Ringer bicarbonate solution containing Ca^{2+} the tissues were blotted, weighed and ashed. Samples from the efflux tubes and dissolved ash were dried on aluminum planchettes and counted in a micro-window continous gas flow counter (Nuclear Chicago D-47). For more detailed experimental procedure and composition of Krebs–Ringer bicarbonate solution see ref. 3.

Each curve of Fig. 1 shows the decline of the log of ^{45}Ca concentration in the uterine horns as they lose radioactivity to the Ca^{2+}-free Krebs–Ringer bicarbonate solution for the first 65 min and then as they lose it at a faster rate to the Ca^{2+}-containing Krebs–Ringer bicarbonate solution. The stimulation of the ^{45}Ca efflux increases with increasing external Ca^{2+} concentrations and saturates at about 2 mM.

In Fig. 2 we have plotted the results of 5 of these sets of experiments in a different way. The increase in the rate of ^{45}Ca efflux Δk (the change in the slope of the efflux curve at 65 min multiplied by 2.3) is plotted as a function of the external Ca^{2+} concentration. Each point is the average of 5 experiments and each verticle bar indicates twice the standard error of the mean. To these points is fitted a calculated curve for the hyperbolic function:

$$\frac{\Delta k}{\Delta k_{max}} = \frac{[Ca^{2-}]}{K + [Ca^{2+}]}$$

where $[Ca^{2+}]$ = the concentration of Ca^{2+} in the efflux solution after 65 min; Δk_{max} = the maximum change in rate of ^{45}Ca efflux = 2.21 h^{-1}; and K = a constant corresponding mathematically to the dissociation constant in the law of mass action expression = $2.7 \cdot 10^{-4}$ M.

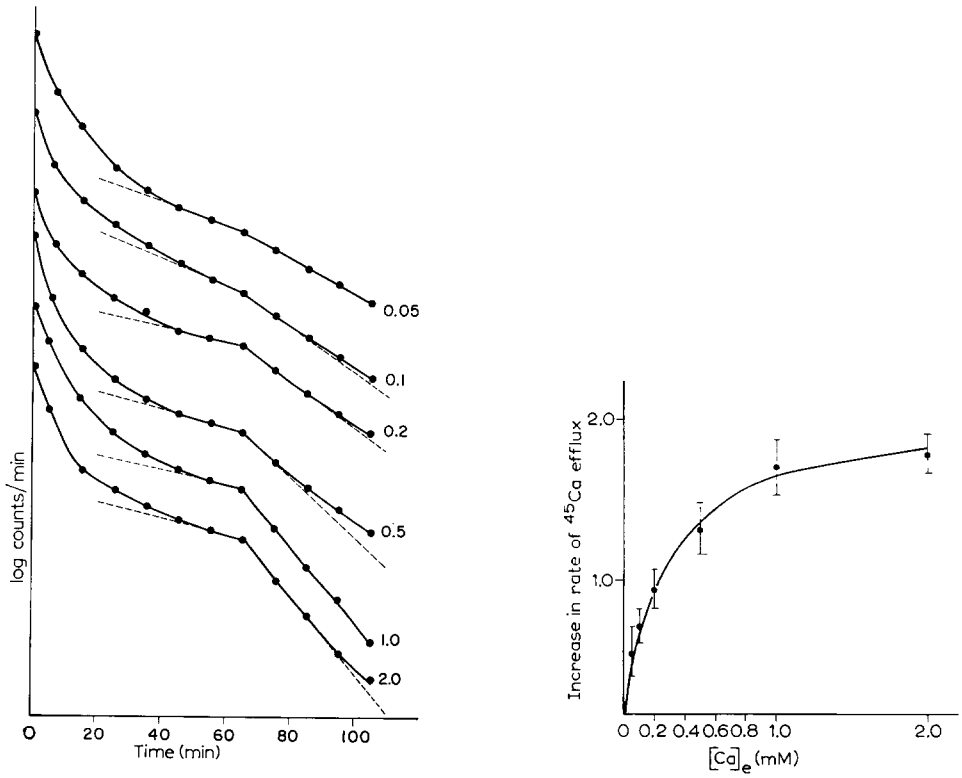

Fig. 1. Efflux curves (counts/min in tissue *vs.* time) from a group of rat uterine horns which were effluxed into Ca^{2+}-free Krebs–Ringer bicarbonate solution for the first 65 min and into Krebs–Ringer bicarbonate solution containing Ca^{2+} for the following 40 min. The number to the right of each curve indicates mmoles Ca^{2+} per l of Krebs–Ringer bicarbonate solution during the last 40 min. For the convenience of showing all Ca^{2+} concentration effects in one figure only the shapes of the curves are shown, not the absolute values. The tissue concentrations of ^{45}Ca at time zero were approximately the same in all uterine horns.

Fig. 2. The increase in rate of ^{45}Ca efflux upon changing from Ca^{2+}-free to Ca^{2+}-containing Krebs–Ringer bicarbonate solution (Δk in h^{-1}) is plotted as a function of the external Ca^{2+} concentration. The verticle bars indicate twice the standard errors of the means. The solid line represents the theoretical curve for the equation $\Delta k/\Delta k_{max} = [Ca^{2+}]/(K + [Ca^{2+}])$. See text for symbols used.

It is of interest to reflect on the possible meaning of the hyperbolic relationship between the increase in ^{45}Ca efflux and the external Ca^{2+} concentration, and on the value of K. BRINK[5] suggests that a hyperbolic relationship between a cellular property (in this case an increased ^{45}Ca efflux) and the external Ca^{2+} concentration reflects the binding of Ca^{2+} to a cellular component (in this case probably the cell membrane). If such a cellular property is linearly related to the concentration of Ca^{2+} bound to the cellular component in question, then the estimated constant K corresponds to the dissociation constant for the binding reaction between Ca^{2+} and the cellular com-

ponent. This interpretation would mean that sites on the intact smooth muscle membranes bind Ca^{2+} and that the dissociation constant of this reaction is $2.7 \cdot 10^{-4}$ M.

Some support for this interpretation is furnished by GENT, TROUNCE AND WALSER[6] who found a dissociation constant of $2.8 \cdot 10^{-4}$ M for the binding of Ca^{2+} to fragmented red blood cell membranes. Results from experiments now in progress in our laboratory make it at least plausible that we are dealing with a membrane phenomenon. In these experiments an artificial phospholipid membrane separates two aqueous solutions. If ^{45}Ca is added to one side of the membrane, the addition of non-labelled Ca^{2+} to the other side of the membrane greatly increases the flux of ^{45}Ca into the solution containing the non-labelled Ca^{2+}. This phenomenon also saturates at about 2 mM.

A measure of Ca^{2+} affinity of smooth muscle cell surfaces is of particular interest since it is widely accepted that a superficially bound Ca^{2+} fraction regulates smooth muscle excitability[7–10]. It has also been proposed that drugs and neurotransmitters modify this Ca^{2+}-mediated regulation by changing membrane affinity for Ca^{2+} (refs. 11, 12).

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