Free Calcium at Rest During "Catch" in Single Smooth Muscle Cells

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Tension and intracellular free calcium concentration ($[Ca^{2+}]_i$) were measured simultaneously in single smooth muscle cells isolated from the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* that were loaded with the fluorescent Ca^{2+} indicator fura-2. Electrical stimulation evoked a transient elevation of $[Ca^{2+}]_i$ associated with a "catch" contraction. During the catch state, however, $[Ca^{2+}]_i$ was effectively at its resting level and was unaffected by 5-hydroxytryptamine, which induced a rapid relaxation from catch. The results indicate that a maintained high $[Ca^{2+}]_i$ is not required for the maintenance of catch tension in intact ABRM and that there was no significant change in $[Ca^{2+}]_i$ upon abolition of catch.

SOME MOLLUSCAN SMOOTH MUSCLES such as the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* exhibit a "catch" contraction associated with an extremely slow relaxation (*1*). The catch contraction allows the muscle to maintain tension for a prolonged period of time with little expenditure of energy, and this highly economical contraction appears to be essential for the survival of bivalve mollusks. Mechanically, the catch state is well characterized by the reduced ability of the muscle to regenerate tension after a sudden reduction in muscle length (quick release) (*2*), and this fact implies that the catch state may involve a slow cycling rate of actomyosin crossbridges. However, the precise mechanism of the catch is still unknown.

A neurotransmitter of the "relaxing" nerve, 5-hydroxytryptamine (5HT), interrupts the catch state and brings about a rapid relaxation (*3*). This effect of 5HT is mediated by an increase in intracellular cyclic adenosine monophosphate (cAMP) concentration (*3*), which may promote phosphorylation of myofilament proteins—for example, paramyosin (*4*), a myosin light chain (*5*), the myosin heavy chain (*6*), or myofilament protein kinases (*7*) in order to produce relaxation.

However, flux studies with $^{45}Ca^{2+}$ showed that 5HT also stimulates Ca^{2+} efflux in intact ABRM (*8*). In addition, our earlier study with an isolated fura-2–loaded cell suspension from the ABRM showed that both 5HT and forskolin, as well as membrane-permeant forms of cAMP, can modulate changes in $[Ca^{2+}]_i$ induced by carbachol or KCl (*9*), implying action through changes in the level of cAMP. These studies and others in which murexide was used (*10*) suggest that changes in intracellular Ca^{2+} can also play a part in the regulation of the catch contraction. Skinned fiber preparations of the ABRM exhibit a catch-like state in high and low Ca^{2+} concentrations. This phenomenon depends on both the ionic strength and the *p*H (*11*), a fact that has hindered the determination of the exact relation between Ca^{2+} and the catch state. We measured $[Ca^{2+}]_i$ during the initiation of and relaxation from catch in single intact ABRM cells loaded with the fluorescent Ca^{2+} indicator fura-2 (*12*) in order to determine the mechanisms of the catch state.

Smooth muscle cells from the ABRM were enzymatically dispersed and loaded with fura-2 as described earlier (*13*). Tension and fluorescence at 511 nm were measured simultaneously in cells loaded with fura-2 by the use of an epifluorescence microscope with a sensitive force transducer system (*14*). The fluorescence of the cell excited at 340 or 380 nm every 25 ms was measured at 511 nm with a photon-counting photomultiplier tube (PMT) for 20 ms of each 25-ms cycle; a Deltascan (Photon-Technology, Inc.) was used for this purpose (Fig. 1).

$[Ca^{2+}]_i$ was calculated point by point from the ratio of the fluorescence at 340 nm to that at 380 nm by use of the equation described by Grynkiewicz *et al.* (*12*). The fluorescence ratios of Ca^{2+}-saturated (R_{max}) and Ca^{2+}-free (R_{min}) fura-2 within the cells were separately determined as follows. Immediately after the measurements of tension and fluorescence, 10 μl of the same batch of cell suspension (containing about 10^4 cells) was put into a small chamber. R_{max} and R_{min} were measured by adding 50 μ*M* (final) digitonin and subsequently 10 m*M* tris-HCl (*p*H 7.4) and 20 m*M* EGTA, respectively. A value of $Kd_{Ca^{2+}}$ (apparent dissociation constant) for fura-2 of 248 n*M* (*12*) was used.

A 0.5-ms single electrical pulse produced a long-lasting tension response. After a quick release (1.5% to 2% of cell length) given during the tension maintenance, the cell was unable to redevelop tension during the released state, indicating that the active state had decayed and that catch state was established (Fig. 2). The stimulus intensity was set to be just above threshold, since the cells became damaged after a strong contraction (similar to that evoked by the second stimulation in Fig. 2B). Hence the mean tension at the peak of contraction (4.7×10^3 kg/m^2) was considerably less than the maximal tension reported for whole ABRM (8×10^4 to 14×10^4 kg/m^2) (*15*). When the experimental chamber was perfused with 5HT (10 μ*M*), a rapid relaxation of catch was observed (Fig. 2B). In the presence of 5HT, the cells showed a "phasic" contraction in response to a subsequent electrical stimulation (Fig. 2A). This behav-

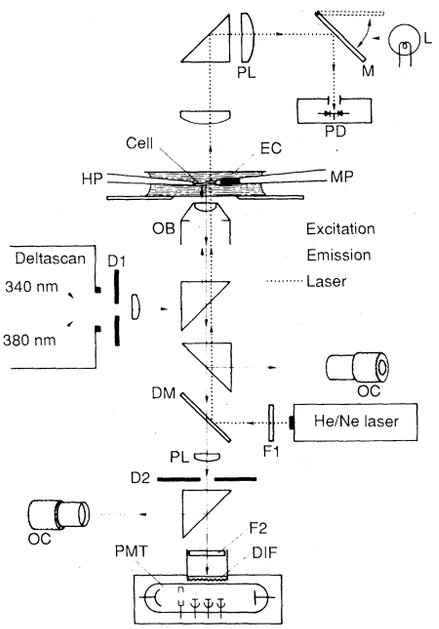

Fig. 1. Schematic experimental arrangement. One milliliter of the suspension of fura-2 loaded cells (containing approximately 100 cells) in artificial seawater (Ca^{2+} concentration, 4 m*M*) was placed in a chamber (EC) on an epifluorescence microscope (Nikon Diaphot) equipped with ultraviolet-compatible optics. Ends of the cell were fixed by suction with two glass micropipettes, one of which, the measurement pipette (MP), was painted black near the tip and was more pliable (compliance <2% cell length) than the other, the holding pipette (HP). The real image of the black-painted tip of the MP was projected by a He/Ne laser (wavelength, 632.8 nm) onto a differential photodiode (PD) (Siemens PX-48), which detected a small elastic displacement of the MP (<2% of the effective cell length) as a measure of tension (*14*). The MP was calibrated for its compliance as described earlier (*14*). The cells were stimulated electrically by using both the pipettes as suction electrodes. All measurements were made at 20°C. D1 and D2, field-limiting diaphragms; DIF, diffuser; DM, dichroic mirror; F1, sharp cut filter (<0.05% transmission below 600 nm); F2, 511 ± 3 nm bandpass filter; L, white light source for visual observation; M, movable mirror; OB, objective; OC, ocular; PL, projection lens.

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ior is consistent with that of the whole ABRM muscle preparation (*1*). In the cell shown in Fig. 2B, however, the second stimulation elicited a stronger contraction associated with maintained $[Ca^{2+}]_i$ and visible damage to the cell.

These contractions were associated with an increase in $[Ca^{2+}]_i$ from a mean resting value of 138 ± 21.3 n*M* (mean ± SE, $n = 6$) to between 500 and 600 n*M*. Both in the absence and presence of 10 μ*M* 5HT, however, the change in $[Ca^{2+}]_i$ was more transient than the tension response (Fig. 2). In the catch contraction, $[Ca^{2+}]_i$ quickly returned to its resting level with a half-time ($t_{1/2}$), which was smaller by an order of magnitude than that of the slow tension decay (Table 1). The application of 5HT during the catch did not affect $[Ca^{2+}]_i$, which was already close to the resting level, even though it brought about a rapid relaxation of force (Fig. 2B). No significant difference was observed between the transient $[Ca^{2+}]_i$ responses upon electrical stimulation in the absence of 5HT and those in the presence of 5HT, despite the marked change in the time course of relaxation (Fig. 2A and Table 1). Thus, our results are conclusive evidence that both the maintenance of tension during catch and the relaxation induced by 5HT are independent of changes in $[Ca^{2+}]_i$ and occur at resting Ca^{2+} concentrations. Other mechanisms involving the cAMP-dependent phosphorylation of the myofilament or the proteins or myofilament protein kinases may control relaxation of the catch state. More recent experiments (*16*) indicate that in cell suspensions there is no change in pH_i on stimulation with carbachol or KCl or on the subsequent addition of 5HT.

A phenomenon similar to the catch state has been reported in vertebrate arterial smooth muscle—that is, the "latch" contraction (*17*). The latch state is defined by the high resistance to stretch and the poor ability of the muscle to shorten actively after an isotonic release. In contrast to the catch contraction, however, $[Ca^{2+}]_i$ is believed to be maintained at slightly higher than resting levels (*17*) in the latch state. Our finding that 5HT does not affect the resting $[Ca^{2+}]_i$ (Fig. 2) is consistent with the results of our earlier study with a fura-2–loaded cell suspension from ABRM (*9*) and is in marked contrast to results obtained in a study by Parker *et al.* (*18*), which showed that, in vertebrate visceral smooth muscle, the resting $[Ca^{2+}]_i$ is markedly decreased by both β-adrenergic agonists and cAMP.

The discrepancy between the present Ca^{2+} measurements and the earlier $^{45}Ca^{2+}$ flux studies in whole ABRM could be due to the presence in the latter of extracellularly bound $^{45}Ca^{2+}$ or by the presence of nonmuscle cells that are responsive to 5HT. Alternatively, Ca^{2+} bound to some intracellular proteins during the catch state may be released by the effect of 5HT and cAMP, with a barely detectable change in $[Ca^{2+}]_i$, particularly if the released Ca^{2+} is extruded quickly by 5HT- and cAMP-activated cell membrane Ca^{2+} pumps (*9*). Alternatively, higher force levels than those used in these experiments might have revealed $[Ca^{2+}]_i$ changes. A measure of Ca^{2+} bound to the myofilaments in intact cells will be needed to test these points.

In conclusion, although an increased free Ca^{2+} is instrumental in initiating the catch state in ABRM muscle, it is not necessary for the maintenance of this state, nor is a change in free Ca^{2+} evident on abolition of catch.

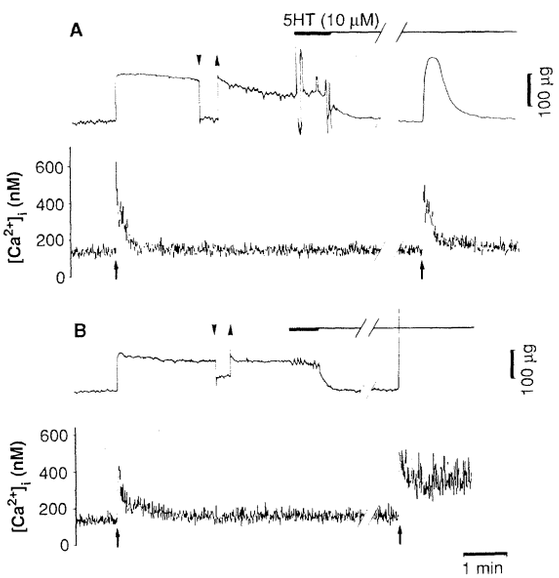

Fig. 2. Records of tension (upper trace) and $[Ca^{2+}]_i$ (lower trace) obtained from two different cells. The cells fixed at both ends were first stretched until the slack was just taken up, then stimulated with gradually increased intensity until a contraction was detected. Arrows indicate a 0.5-ms electrical pulse. Arrowheads indicate a quick release by 1.5% to 2% of cell length and a subsequent restretch to the original length. Bars above the tension traces indicate the presence of 10 μ*M* 5HT (thickened bar, perfusion of 5HT), and the second stimulation was given to the cells in the presence of 5HT. In (**A**) the relaxation process induced by 5HT is obscured by a large perfusion artifact, whereas in (**B**) it can be seen more clearly owing to a reduced perfusion rate. The second stimulation in (B), however, caused an irreversible contracture. Quick release and restretch were performed by the piezo-electric bimorph ceramic on which the holding pipette was mounted. 5HT (10 μ*M*) in artificial seawater was injected into the experimental chamber at the rate of 1 to 2 ml/min by a peristaltic pump, while an excess amount of the bathing solution was continuously withdrawn by an aspirator.

Table 1. Peak values and half-times for tension and $[Ca^{2+}]_i$ responses ($\Delta[Ca^{2+}]_i$) in the absence (catch contraction) and presence (phasic contraction) of 10 μ*M* 5HT. Values are means ± SE.

Contraction	Tension		Δ $[Ca^{2+}]_i$		*n*
	Peak ($\times 10^3$ kg/m^2)	$t_{1/2}$ (s)	Peak (n*M*)	$t_{1/2}$ (s)	
Catch	4.72 ± 0.69	197 ± 40	455 ± 36	9.1 ± 2.2	6
Phasic	4.88 ± 0.93	15.5 ± 1.9	430 ± 44	7.9 ± 1.0	6

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