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Mechanical stretch increases intracellular calcium concentration in cultured ventricular cells from neonatal rats

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Summary. We investigated the effects of mechanical stretch on intracellular calcium concentration ($[Ca^{2+}]_i$) of cultured neonatal rat ventricular cells using microfluorometry with fura-2. Myocytes were cultured on laminin-coated silicon rubber and stretched by pulling the rubber with a manipulator. Myocytes were either mildly stretched (to less than 115% of control length), moderately so (to 115%–125% of control length), or extensively (to over 125% of the control length). "Quick stretches" (accomplished within 10 s) of moderate to extensive intensities produced a large transient increase of $[Ca^{2+}]_i$ in the early phase of stretch (30 s–2 min), followed by a small but sustained increase during the late phase of stretch (5–10 min). The initial transient increase in $[Ca^{2+}]_i$ after the "quick stretch" was preserved in the presence of gallopamil (10^{-7} M) or ryanodine (10^{-5} M), but was absent in Ca^{2+}-free medium or in the presence of gadolinium (10^{-7} M). The late or steady state $[Ca^{2+}]_i$ increase was observed in the presence of gadolinium, gallopamil, or ryanodine but was abolished in Ca^{2+}-free medium. A steady-state increase in $[Ca^{2+}]_i$ was also evoked by "slow stretch" in which cells were slowly pulled to the final length within 1–2 min. As the presence of external Ca^{2+} was indispensable, increased trans-sarcolemmal Ca^{2+} influx appears to be involved in both initial and steady-state increases in $[Ca^{2+}]_i$. The initial increase in $[Ca^{2+}]_i$ after the "quick stretch" can be attributed to the activation of gadolinium-sensitive, stretch-activated channels.

Key words: Ca^{2+} transient — Fura-2 — Mechanical stretch – Cultured neonatal rat ventricular cells — Stretch-activated channel

Introduction

Electrical excitation of cardiac cells produces an increase in intracellular calcium concentration ($[Ca^{2+}]_i$) which, in turn, triggers the development of contraction. Changes in membrane tension caused by contraction or by passive mechanical stretch elicit alterations in the ionic currents of cardiac cells [1–3] and alter the membrane potential [4–7]. This phenomenon, termed "contraction-excitation feedback", is attributed to alterations in $[Ca^{2+}]_i$ that occur secondarily to membrane stretch [8]. Two possible mechanisms of increase in $[Ca^{2+}]_i$ have been proposed. One is the opening of stretch-activated channels (SACs) that carry cations such as Na^+, K^+, and Ca^{2+} [9]. The possible involvement of SACs in stretch-induced increase in $[Ca^{2+}]_i$ was proposed in guinea-pig ventricular myocytes [10], in rat atrial cells [11], and in cultured chick heart cells [9]. The second mechanism is the enhancement of the L-type Ca^{2+} current observed in rabbit ventricular myocytes subjected to hypotonic stress or inflation of the cell by positive pressure applied through the patch electrode [12]. Using fura-2 fluorescent microscopy, we tested the mechanisms of mechanically induced increase of $[Ca^{2+}]_i$ in neonatal rat ventricular cells cultured on a thin silicon film. Stretching the cells by pulling the silicon film, we observed a large transient increase in $[Ca^{2+}]_i$, followed by a sustained increase in $[Ca^{2+}]_i$ during maintained stretch. The early transient increase in $[Ca^{2+}]_i$ appeared to be due to Ca^{2+}-influx through gadolinium-sensitive, stretch-activated channels (SACs). The sustained steady-state increase in $[Ca^{2+}]_i$ was dependent on external Ca^{2+} and was resistant to gadolinium, gallopamil, and ryanodine (blockers of SACs, L-type Ca^{2+} channels, and Ca^+ release channels in sarcoplasmic reticulum, respectively). A preliminary report on this subject has appeared elsewhere [13].

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Materials and methods

Cells harvested from neonatal Wistar rat ventricles (1 to 5 days old) were cultured for 5 days, according to the method of Tatsukawa et al. [14], on a strip of laminin-coated autofluorescence-free silicon rubber (thickness, 0.15 mm; size, 5 × 40 mm; Fuji Systems, Yokohama, Japan). Most cell aggregates (>95%) showed slow spontaneous activity at rates <0.2 Hz, with the rest of the aggregates (<5%) remaining quiescent. The cells were loaded with fura-2/AM (Wako Pure Chemicals Osaka, Japan), using a method described previously [14].

Cell aggregates (loaded with fura-2) were placed on the stage of a dual-wavelength fura-2 microfluorometer (CAM 200; Nihonbunnko, Tokyo, Japan), and field-stimulated at 0.5 Hz or left without stimulation. Systolic $[Ca^{2+}]_i$ (sCa) and diastolic $[Ca^{2+}]_i$ (dCa) of Ca^{2+} transients in stimulated cells, and the resting $[Ca^{2+}]_i$ (resting Ca) of quiescent cells were measured at 22°C–26°C. After the subtraction of background autofluorescence signals in the observation field (80 × 80 μm) with no distribution of cells, the fluorescent ratio (R340/380) was calculated by dividing the signal at excitation wavelength of 340 nm with that at 380 nm. The time to peak and the half decay time of the Ca^{2+} transient were analyzed, as described [14]. All statistical analyses of $[Ca^{2+}]_i$ were made by averaging 20 Ca^{2+} transients for sCa and dCa in stimulated cells and by averaging 20 $[Ca^{2+}]_i$ values (sampled at 2-s intervals) for "resting Ca" in non-stimulated quiescent cells.

Ventricular cells cultured on silicon rubber were stretched mildly (to less than 115% of control length), moderately (to 115%–125% of control length), and extensively (to over 125% of the control length), by pulling the silicon rubber with a custom-made micromanipulator (modified from Model A-3; Narishige, Tokyo, Japan). The stretch was applied along the longitudinal axis of the strip of silicon rubber. The speed of stretch was controlled manually in two ways: "quick" and "slow". "Quick stretch" was applied to the rubber to reach a certain desired length within 10 s and kept at that length for up to 10 min, while "slow stretch" was accomplished much more slowly, i.e., taking 1–2 min to attain the desired length. The intensity of stretch was measured from the image of the cells stored on VCR tapes, utilizing an image analysis system (Nexus 600; Nexus, Tokyo, Japan) and was expressed as a percentage of the control length.

Cell aggregates on the silicon rubber were perfused with Tyrode's solution of the following composition (in mM): NaCl 135, KCl 5.4, $MgSO_4$ 1, $CaCl_2$ 0 or 1.8, NaH_2PO_4 1, $NaHCO_3$ 3, glucose 10, HEPES 10, with pH adjusted to 7.4 by adding NaOH. In some experiments, ryanodine, an inhibitor of Ca^{2+} release channels of sarcoplasmic reticulum (Agri System International; Windgap, PA, USA), gallopamil, a blocker of sarcolemmal Ca^{2+} channels (a kind gift from Taisho Pharmaceutical, Osaka, Japan) or gadolinium chloride, a blocker of sarcolemmal stretch-activated channels (Sigma, St Louis, MO, USA) were added to the Tyrode's solution.

All values were expressed as means ± SEM. For the statistical analyses, one way analysis of variance (ANOVA) was used. When a suitable F-statistic was obtained using ANOVA, mean values were compared using Duncan's multiple range test; $P < 0.05$ was considered significant.

Results

$[Ca^{2+}]_i$ changes elicited by "quick stretch"

Figure 1 shows representative effects of "quick stretch" (accomplished within 10 s) of moderate intensity (118% of control length) on fluorescence signals at excitation wavelengths of 340 and 380 nm (F340 and F380) and their ratio (R340/380) in a rat ventricular cell aggregate cultured on silicon rubber and stimulated at 0.5 Hz. The stretch produced a marked but transient increase in R340/380. In the early phase (30 sec–2 min after application of stretch), the diastolic $[Ca^{2+}]_i$ (dCa) increased markedly, beyond the systolic $[Ca^{2+}]_i$ (sCa) of the control condition. The maximum value of dCa was estmated to be around 2000 nM, using the "in vitro" calibration methods described by Tatsukawa et al. [14]. This increase was followed by a sustained and much lower level of $[Ca^{2+}]_i$ increase in the late (or steady-state) phase (5–10 min after application of stretch). Slightly enhanced $[Ca^{2+}]_i$ signals seen in the late phase were restored completely to the control (pre-stretch) level after release of the stretch (extreme right in Fig. 1).

A similar initial increase in dCa (more than 20% increase in the value of R340/380) was observed in five of seven preparations subjected to "quick stretch" with moderate to extensive intensities. Average increases in dCa in responding preparations were found to be 85 ± 34% (the value of R340/380 increased from 0.62 ± 0.03 before, to 1.15 ± 0.16 after stretch, $n = 5$). Measurement of sCa was difficult because of loss of Ca^{2+} transient often encountered in the very beginning of the stretch (see Fig. 1). As there were symmetrical changes in fluorescence signals at excitation wavelengths of 340 nm and 380 nm (top two traces in Fig. 1), the change in $[Ca^{2+}]_i$ that occurred during stretch was not an artifact, e.g., due to some mechanical movement of the preparation. The intense increase in $[Ca^{2+}]_i$ seen in the early phase was observed only with "quick stretch of moderate to extensive intensities (not with mild inten-

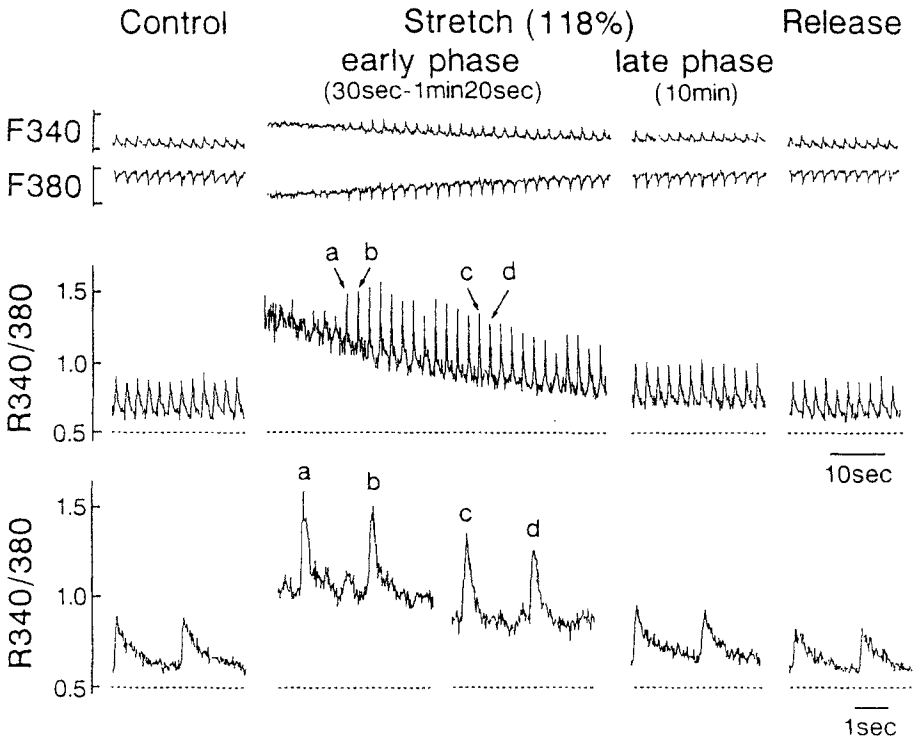

Fig. 1. Effects of "quick stretch" of moderate intensity (118% of control length) and its release on fluorescence signals of fura-2 (F340 and F380) and their ratio (R340/380), which indicate intracellular Ca^{2+} concentration $[Ca^{2+}]_i$. The stretch produced a marked transient increase in R340/380 (early phase), followed by a sustained or steady state increase (late phase). The *bottom panel* shows enlarged Ca^{2+} transients (*designated by the same letter in the middle panel*). The apparently lower peak amplitude in *a* in the *middle panel* than that in the bottom panel is an artifact due to lower sampling frequency in the middle panel. *Dotted lines* are drawn for handy comparison of the change in Ca^{2+} transients, in this and subsequent *Figs. 2 through 4*

sity)", whereas the $[Ca^{2+}]_i$ increase in the late or steady-state phase was provoked irrespective of quick or slow stretches of any intensity.

To elucidate the mechanism(s) of initial increase in $[Ca^{2+}]_i$ caused by "quick stretch", we examined the effects of external Ca^{2+} concentration ($[Ca^{2+}]_o$) on this increase of $[Ca^{2+}]_i$, using "non-stimulated quiescent cell aggregates". Figure 2A shows the effects of "quick stretch" of moderate intensity on the $[Ca^{2+}]_i$ of a non-stimulated, quiescent cell aggregate in the presence of 1.8mM $[Ca^{2+}]_o$. In this series of experiments, the initial phase of $[Ca^{2+}]_i$ increase was relatively small, though clearly visible, compared to that seen in electrically stimulated preparations (see Fig. 1). In the initial phase of stretch, more than 20% increase in the ratio of R340/380 was observed in three of seven preparations (the value of R340/380 increased from 0.53 ± 0.04 before, to 0.67 ± 0.04 after stretch; $n = 3$). On the other hand, as shown in Fig. 2B, the ratio remained unchanged in all preparations tested under conditions of nil $[Ca^{2+}]_o$ (0.58 ± 0.06, control versus 0.59 ± 0.05; $n = 7$). These results suggest that the influx of external Ca^{2+} is pivotal for the stretch-induced increases in $[Ca^{2+}]_i$.

We then examined the mechanisms of the initial increase in $[Ca^{2+}]_i$, using some channel blockers. Included were ryanodine (to block Ca^{2+} release channels of sarcoplasmic reticulum), gallopamil (to block sarcolemmal Ca^{2+} channels), and gadolinium (to block sarcolemmal stretch-activated channels). Figure 3 shows representative effects of ryanodine, gallopamil, and gadolinium, on $[Ca^{2+}]_i$ changes induced by a stretch of moderate intensity (stimulated preparations). The "early" increase in $[Ca^{2+}]_i$ was still found in the presence of 10^{-5}M ryanodine (Fig. 3A) or 10^{-7}M gallopamil (Fig. 3B), but not in the presence of 10^{-5}M gadolinium (Fig. 3C). However, the "early" increase in $[Ca^{2+}]_i$ in the presence of either ryanodine or gallopamil tended to be attenuated, compared to that recorded in the absence of these drugs (Fig. 1). It is noteworthy that the late or steady-state increase in $[Ca^{2+}]_i$ was still recognized after abolition of the "early" increase by gadolinium. Essentially the same findings were obtained in all three other preparations tested with ryanodine, two other preparations with gallopamil (10^{-6}M), and five other preparations with gadolinium. Release of the stretch restored both systolic and/or diastolic levels of $[Ca^{2+}]_i$ to the control levels (see extreme right panels of Fig. 3).

Effects of "slow stretch" on $[Ca^{2+}]_i$

The late or steady-state increase of $[Ca^{2+}]_i$ was provoked by "slow stretch", i.e., even in the absence of a preceding marked increase in $[Ca^{2+}]_i$ caused by "quick stretch". Figure 4 shows representative effects of 'slow stretch" of various intensities on Ca^{2+} transients. It is clear that the peak systolic levels of $[Ca^{2+}]_i$ (sCa) increased, in a stretch intensity-dependent manner. The results obtained from seven preparations are summa-

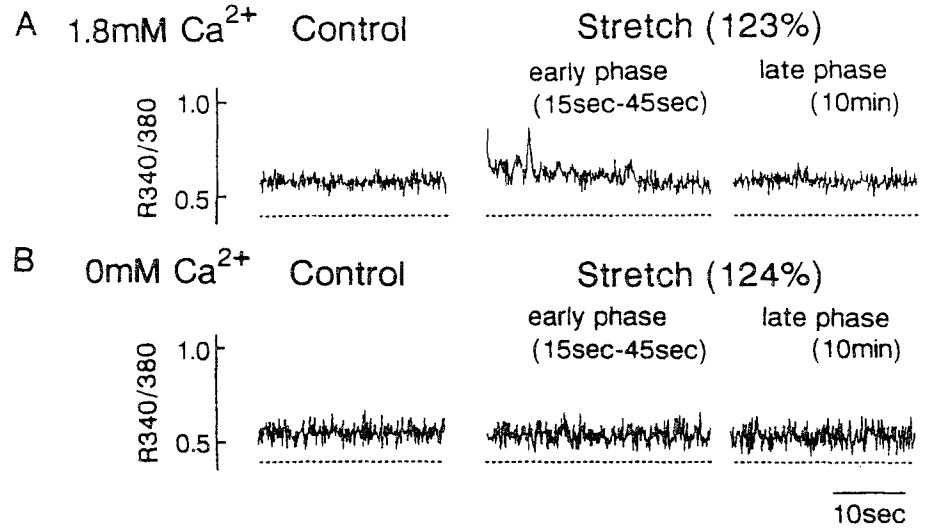

Fig. 2A,B. Effects of external Ca^{2+} concentration (1.8 mM in **A** versus nominally free in **B**) on $[Ca^{2+}]_i$ change induced by "quick stretch" of moderate intensity (123% and 124% of control length, respectively). These experiments were done using non-stimulated, quiescent cell aggregates

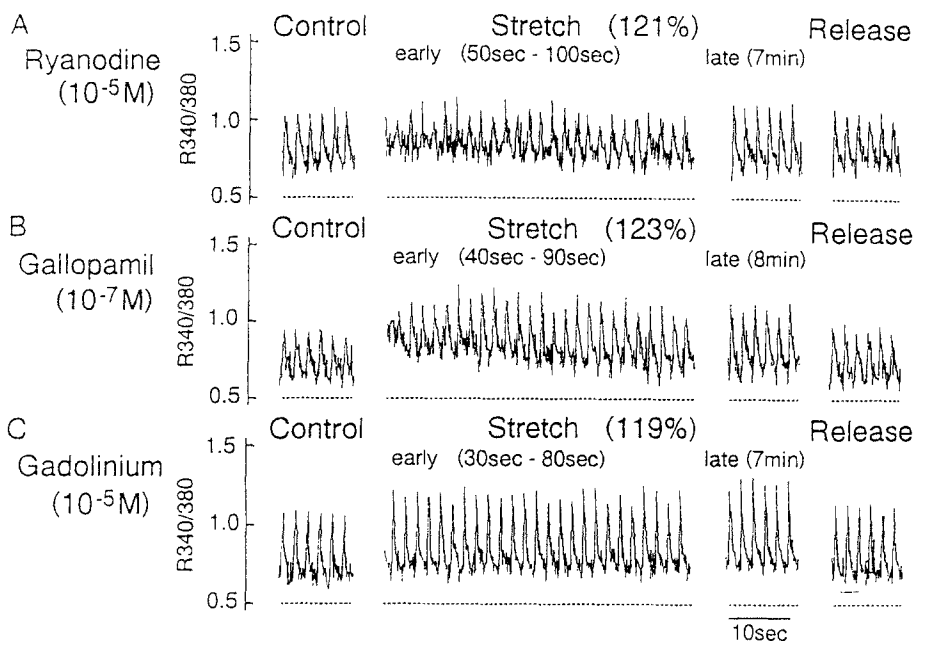

Fig. 3A–C. Effects of allegedly specific blockers for Ca^{2+} release channel of sarcoplasmic reticulum (ryanodine), sarcolemmal Ca^{2+} channel (gallopamil), and sarcolemmal stretch-activated channel (gadolinium), on the $[Ca^{2+}]_i$ before, during, and after application of "quick stretch" of moderate intensities (119%–123% of control length). Records **A**, **B**, and **C** are from three different cell aggregates. Note that gadolinium abolished the early transient increase in $[Ca^{2+}]_i$

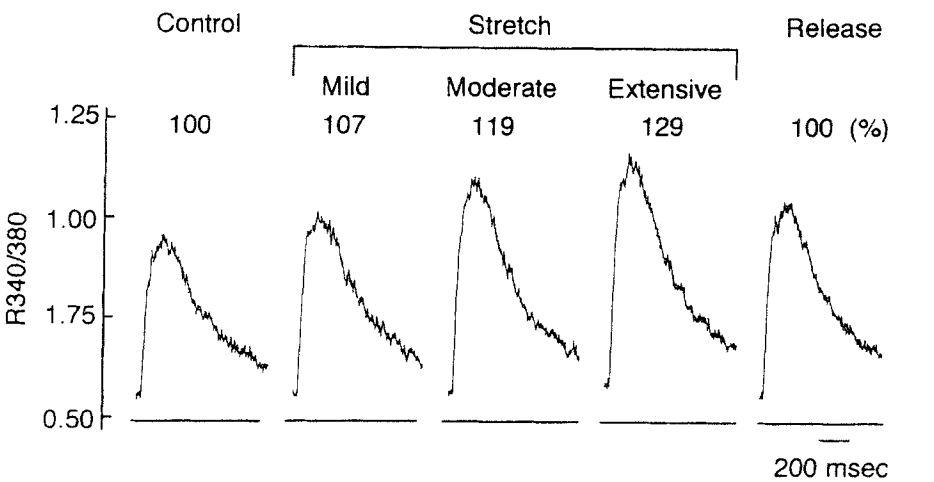

Fig. 4. Effects of "slow stretch" (accomplished within 1–2 min) of mild, moderate, and extensive intensities on Ca^{2+} transients. After the taking of a control record (*extreme left*), the preparation was stretched slowly with increasing intensity, stepwise, and then released (*extreme right*). Each Ca^{2+} transient was recorded about 5 min after attaining the respective length, indicated on the *top*

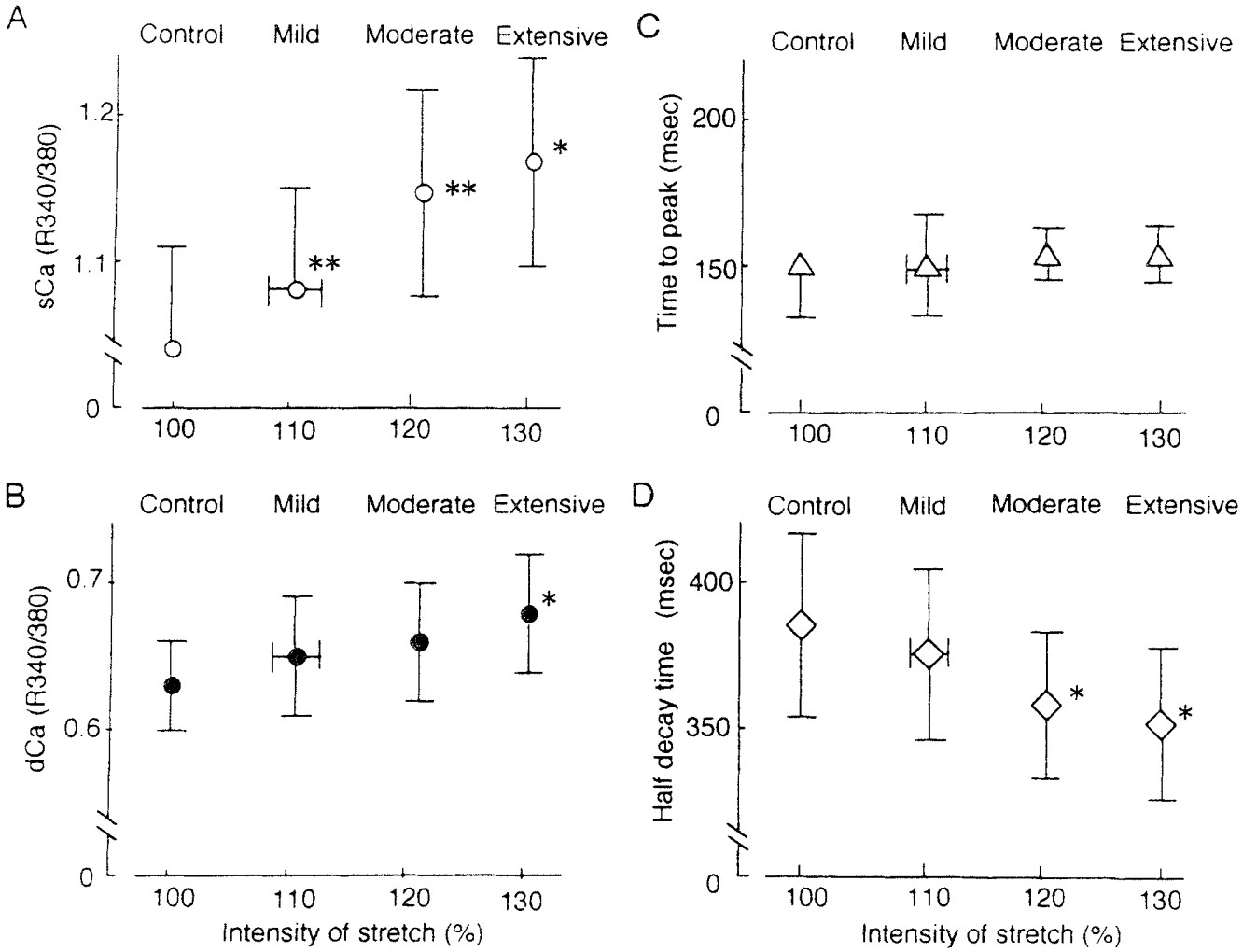

Fig. 5A–D. Summary of the effects of "slow stretch" on systolic $[Ca^{2+}]_i$ (**A**, *sCa*); **B** diastolic $[Ca^{2+}]_i$ (*dCa*), **C** time to peak, and **D** half decay time of Ca^{2+} transient. *Vertical and horizontal bars* indicate SEM and are abbreviated when they are smaller than the size of the symbol. * $P < 0.05$; ** $P < 0.01$, significant differences from control value ($n = 7$)

rized in Fig. 5. The levels of sCa significantly increased in proportion to the intensity of stretch (Fig. 5A), while dCa increased significantly only with extensive stretch (Fig. 5B). Both dCa and sCa remained unchanged for as long as 40 min when preparations were kept in their slack length ($n = 3$). The effects of slow stretch on rising and decaying time courses of Ca^{2+} transients were also analyzed. The half decay time of Ca^{2+} transients was significantly ($P < 0.05$) shortened in response to stretches with moderate to extensive intensities (Fig. 5D), while the time to peak of the Ca^{2+} transient remained unaltered (Fig. 5C).

Finally we examined the effect of altered $[Ca^{2+}]_o$ on $[Ca^{2+}]_i$ changes produced by "slow stretch" of various intensities, using non-stimulated quiescent cell aggregates. Figure 6 summarizes the results of such experiments. Resting $[Ca^{2+}]_i$ was measured during "slow stretch" of mild, moderate, and extensive intensities, in the presence (1.8 mM) or absence (0 mM) of external Ca^{2+}. The resting $[Ca^{2+}]_i$ under control (pre-stretch) condition and in the presence of 1.8 mM $[Ca^{2+}]_o$ was significantly higher than that of 0 mM $[Ca^{2+}]_o$ ($n = 4$; $P < 0.05$). In the presence of 1.8 mM $[Ca^{2+}]_o$, the stretches increased the resting $[Ca^{2+}]_i$ and this increase was significant with moderate stretch, but not with mild stretch. On the other hand, in the absence of external Ca^{2+}, stretches of any intensity did not increase the resting $[Ca^{2+}]_i$. Data were missing for extensive stretch in the presence of 1.8 mM $[Ca^{2+}]_o$ because the stretch always provoked spontaneous contractions and the resting $[Ca^{2+}]_i$ could not be registered. These findings suggest that the influx of external Ca^{2+} is likely to be responsible for the increase in $[Ca^{2+}]_i$ caused by "slow stretch", as was the case for "quick stretch".

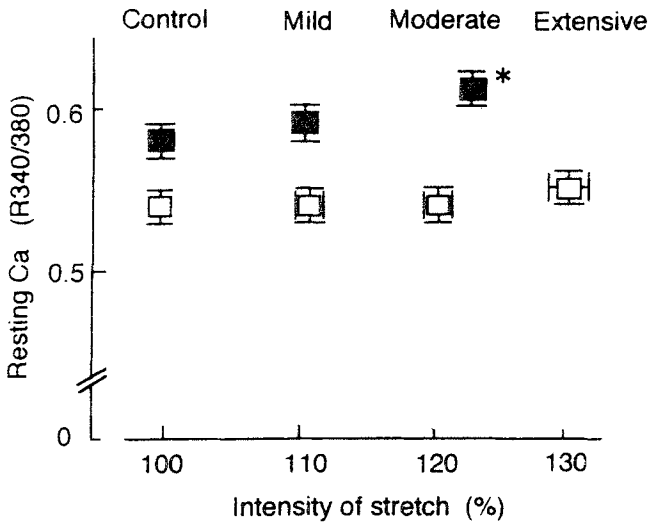

Fig. 6. Effects of "slow stretch" of various intensities on the resting $[Ca^{2+}]_i$ measured in the presence (1.8 mM, *black symbols*) and absence (0 mM, *white symbols*) of external Ca^{2+}. The tests were done using non-stimulated quiescent cell aggregates. $^*P < 0.05$, significant difference from the control value at 1.8 mM Ca^{2+} ($n = 4$ for 1.8 mM Ca^{2+}; $n = 5$ for 0 mM Ca^{2+})

Discussion

A large and transient increase in $[Ca^{2+}]_i$ after "quick stretch"

Ionic channels activated by mechanical stretch exist in cardiac cells [1, 2, 9], as well as in other type of cells [15, 16]. Stretch-activated channels (SACs) in cardiac tissue are suggested to be responsible for the evolution of arrhythmias in animals placed under certain pathologic conditions [4]. Using cultured embryonic chick heart cells, Sigurdson et al. [9] demonstrated that Ca^{2+} flows into the cells via Ca^{2+}-permeable SACs. Mechanical stretch of single guinea-pig ventricular myocytes induced a large increase in the resting $[Ca^{2+}]_i$; this was reversed by streptomycin, a blocker of SACs [17, 18].

In the present study, we observed that $[Ca^{2+}]_i$ (sCa and/or dCa) of cultured neonatal rat ventricular myocytes was increased rapidly and markedly after "quick stretch" and that the increased $[Ca^{2+}]_i$ decayed during the maintained stretch. Similar large, transient increases in $[Ca^{2+}]_i$ were reported in guinea-pig ventricular myocytes stretched mechanically with a pair of carbon fibers [10, 17, 19].

Interestingly, Ca^{2+} transient was small or absent at the very beginning of "quick stretch" (Fig. 1). Similar decreases in Ca^{2+} transient accompanied by a marked depolarization were noted in single guinea-pig ventricular cells subjected to mechanical stretch [10]. Thus, the loss of the Ca^{2+} transient seems to be due to increases in the threshold for action potential initiation, secondary to membrane depolarization.

The stretch-induced increase in $[Ca^{2+}]_i$ in ventricular myocytes was sensitive to external Ca^{2+} concentration and was reversed by the application of streptomycin, a blocker of SACs [16]. In these studies, the initial large increase in $[Ca^{2+}]_i$ was observed in only 40% of the cells tested. The incidence was less than that we observed in electrically stimulated preparations (70%) and tantamount to the incidence seen in quiescent, nonstimulated preparations (40%). The presence or absence of a large and transient increase in $[Ca^{2+}]_i$ may be determined by the density of the functional SACs in these cells. Conditions of cell isolation and/or cell culture may have affected the density of the SACs differently [9].

In contrast, when single ventricular cells from rats and guinea-pigs were mildly stretched (to about 110% of initial length), the amplitude of contraction increased immediately after the stretch, with little accompanying change in the Ca^{2+} transient [20, 21], thereby suggesting an increase in Ca^{2+}-sensitivity of the contractile proteins. This observation is in accordance with ours, in that a comparable "quick stretch" with mild intensity (<115%) did not produce the initial large increase in $[Ca^{2+}]_i$.

The initial phase of $[Ca^{2+}]_i$ increase was observed in non-stimulated quiescent cells at physiological $[Ca^{2+}]_o$ (1.8 mM), but was hardly seen with nil $[Ca^{2+}]_o$, suggesting a contribution of enhanced Ca^{2+}-influx (Fig. 2). However, the increase of $[Ca^{2+}]_i$ seen in non-stimulated preparations (Fig. 2) tended to be small compared to that encountered in stimulated preparations (Figs. 1 and 3). One possible explanation could be that, in addition to the activation of SACs, the stretch increased Ca^{2+} influx through voltage-gated Ca^{2+} channels [12] in stimulated preparations, but not in non-stimulated preparations.

The initial increase in $[Ca^{2+}]_i$ was observed in the presence of ryanodine or gallopamil, but not in the presence of gadolinium, a trivalent cation which blocks SACs [22]. Although gadolinium blocks L-type Ca^{2+} channels as well [23], blockade of the Ca^{2+} current may not be the cause of suppression of the initial increase in $[Ca^{2+}]_i$, as the authentic Ca^{2+} antagonist gallopamil failed to abolish the stretch-activated increase in $[Ca^{2+}]_i$. Streptomycin, another blocker of SACs, reversed large stretch-induced increases in resting $[Ca^{2+}]_i$ in single guinea-pig ventricular myocytes [17]. Thus, the SACs could be the most likely candidate for the pathway of Ca^{2+} entry in this early phase of stretch. Calcium ions may have directly passed the SACs, or sodium ions may have passed this channel to be exchanged with Ca^{2+} via the Na^+-Ca^{2+} exchange mechanism, because the SACs are sorted into "non-specific cation" channels [9]. The $[Ca^{2+}]_i$ increase encountered immediately after stretch was found only with "quick stretch with moderate to extensive intensities" and not with mild intensity. It is

assumed that a relatively strong and quick stretch is a prerequisite for the activation of particular gadolinium-sensitive SACs.

Steady-state increase in $[Ca^{2+}]_i$ during sustained stretch

The initial marked increase in $[Ca^{2+}]_i$ was followed by late (or steady-state) small increases in $[Ca^{2+}]_i$ that lasted as long as the stretch was maintained. Similar increases in $[Ca^{2+}]_i$ were also observed when the preparation was slowly stretched (accomplished within 1–2 min), in that early transient increase in $[Ca^{2+}]_i$ observed with quick stretch was not evident. The mechanism of $[Ca^{2+}]_i$ increase in the late (or steady-state) phase seems to differ from that of the initial phase. The initial transient increase in $[Ca^{2+}]_i$ was gadolinium-sensitive. In contrast, the late or steady-state increase in $[Ca^{2+}]_i$ was not modified by either gadolinium, gallopamil, or ryanodine (Fig. 3). These findings imply that increases in $[Ca^{2+}]_i$ in the late phase may not mediated by the activation of either SACs, sarcolemmal calcium channels, or Ca^{2+} release channels of sarcoplasmic reticulum. Similar steady-state increases in $[Ca^{2+}]_i$ or contraction were noted in ventricular papillary muscles of the ferret heart [24] and in single guinea-pig ventricular myocytes [10, 18, 21]. No increase in $[Ca^{2+}]_i$ was found in the absence of external Ca^{2+} in our study, which indicates that the inflow of external Ca^{2+} is responsible for the increase in $[Ca^{2+}]_i$ in the late phase, as was the case for the early phase. The mechanism of Ca^{2+} inflow in the late phase is unclear. The stretch-activated Cl^- channel noted in rabbit nodal cells [8] could be one possibility, provided that the Cl^- channels were indeed present in cultured neonatal rat ventricular cells and that the channels were activated by the stretch we applied; the resultant depolarization of the cell membrane may lead to an increase in $[Ca^{2+}]_i$ because of the voltage-dependent nature of the Na^+-Ca^{2+} exchange system [25].

Our findings that the half decay time of the Ca^{2+} transient was shortened by increasing the intensity of "slow stretch" (Fig. 5D) is in agreement with findings in ferret ventricular muscles [24], but not with findings in isolated ventricular myocytes from guinea-pigs [21]. Increases in sensitivity to Ca^{2+} of myofilaments and/or enhanced Ca^{2+} uptake by the sarcoplasmic reticulum were suggested to be involved in the shortening of the decay phase of the Ca^{2+} transient [24].

In summary, we have shown that stretching the cell aggregates of cultured neonatal rat ventricular cells increased $[Ca^{2+}]_i$ via two different mechanisms. "Quick stretch" induced a large and transient increase in $[Ca^{2+}]_i$. A gadolinium-sensitive SAC is suggested to be involved. In the later stage of "quick stretch' and in any phase of "slow stretch", a small but sustained increase in $[Ca^{2+}]_i$ was recognized. The mechanism of this sustained increase in $[Ca^{2+}]_i$ is unclear; the stretch-induced membrane depolarization noted in various preparations [5, 8] could be a possibility. As gadolinium was effective in suppressing ventricular arrhythmias induced by mechanical stretch [26], it could be that an increase in $[Ca^{2+}]_i$ via SACs may underlie some types of ventricular tachyarrhythmias [27]. Chemicals that block this specific channel in the heart would be candidates for a new class of antiarrhythmic drugs.

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