Restricted Propagation of Cytoplasmic Ca^{2+} Oscillation into the Nucleus in Guinea Pig Cardiac Myocytes as Revealed by Rapid Scanning Confocal Microscopy and Indo-1

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ABSTRACT Two-dimensional images of cytoplasmic and nuclear free Ca^{2+} movements in cardiac myocytes were obtained at 67-msec intervals using a Ca^{2+}-sensitive fluorescence probe, indo-1, and a rapid scanning confocal laser microscope, Nikon RCM8000. Isolated guinea pig ventricular cells were loaded with indo-1 and stimulated at 0.5 Hz through patch pipettes. On stimulation, nuclear Ca^{2+} concentration ($[Ca^{2+}]$) was observed to rise and fall following cytoplasmic $[Ca^{2+}]$ with an obvious delay. Application of isoproterenol significantly increased the peak $[Ca^{2+}]$ on stimulation in both the cytoplasm and nucleus with no substantial change in the basal $[Ca^{2+}]$; the increase in peak $[Ca^{2+}]$ produced by application of isoproterenol was larger in the cytoplasm than in the nucleus. Under a low $[Na^+]$ condition, the basal $[Ca^{2+}]$ was increased from the control values in both the cytoplasm and nucleus; no difference in basal $[Ca^{2+}]$ was observed between the two regions. The increase in peak $[Ca^{2+}]$ by low $[Na^+]$ in the cytoplasm was significantly larger than that in the nucleus. When the cells were voltage clamped at 0 mV for 3 sec, no difference in the steady state $[Ca^{2+}]$ was observed between the cytoplasm and nucleus. Nuclear $[Ca^{2+}]$ was also observed to increase following a Ca^{2+} wave, a local increase in $[Ca^{2+}]$ propagating within the cytoplasm, with a delay. Thus, we demonstrated in isolated myocardial cells that cytoplasmic Ca^{2+} movements, although hampered by the nuclear envelope, are propagated into the nucleus, a mechanism through which factors affecting cytoplasmic Ca^{2+} may influence intranuclear events.

Keywords: Confocal microscopy, Calcium ion, Indo-1, Myocardium, Nucleus

Intracellular Ca^{2+} is known to be involved in regulation of various cellular activities such as cell contraction, secretion, differentiation and proliferation. An increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]$) was also shown to stimulate DNA synthesis and gene expression, suggesting that nuclear Ca^{2+} may play important roles in these cellular functions (reviewed in ref. 1). Investigating this subject would be difficult without direct measurement of nuclear $[Ca^{2+}]$ under various conditions. Development of Ca^{2+}-sensitive fluoroprobes and their use in combination with confocal microscopy have been shown by many biologists to be remarkably powerful for obtaining two-dimensional high spatial resolution images of intracellular $[Ca^{2+}]$ in various cell types (1–7). Several reports have dealt with nuclear and cytoplasmic $[Ca^{2+}]$. Some workers postulated that the regulation of nuclear $[Ca^{2+}]$ is separate from that of the cytoplasm (5, 6, 8), while others attribute the difference to the nuclear envelope restricting passive diffusion of cytoplasmic Ca^{2+} into the nucleus (9, 10).

Even fewer studies have dealt with nuclear Ca^{2+} in myocardial cells. As the nuclei of isolated myocardial cells are cylindrically shaped with a diameter of a few μm, confocal microscopic observations are desirable for accurate discrimination of cytoplasmic and nuclear Ca^{2+}. However, the cytoplasmic $[Ca^{2+}]$ of myocardial cells changes in the time scale of milliseconds, which is beyond the time resolution of most currently available confocal microscopes to obtain two dimensional images. Thus, most of the confocal images of Ca^{2+} movements in myocardial cells were either obtained at intervals of more than 125 msec or line scanned (11–13). Recently, Minamikawa et al. (14) obtained images of cytoplasmic and nuclear $[Ca^{2+}]$ in cultured myocardial cells at inter-

vals of 83 msec by limiting the area of scanning (frame-scanning).

Recently, a UV-applicable video rate (30 frames/sec) scanning confocal microscope (RCM8000) in which a resonant galvanometer mirror is used for fast horizontal scans was designed and developed by Nikon Corporation (Tokyo) (15). The principles of this system are almost the same as those of the system developed by Tsien's group (16, 17). Kawanishi et al. (15) obtained ratio-images of Ca^{2+} responses induced by hormonal stimulations in cultured hepatocytes using a prototype of RCM8000 and indo-1. In our previous study, we applied this microscope to spontaneously beating cultured cardiac myocytes to obtain two-dimensional confocal images of intracellular Ca^{2+} movements at 33-msec intervals (18). Using fluo-3 as the fluoroprobe, we could observe two types of Ca^{2-} movements which were characterized pharmacologically, and we also observed that fluorescent signals from the nucleus rise and fall following that of the cytoplasm with an obvious delay. In the present study, we applied RCM8000 and indo-1 on freshly isolated guinea pig ventricular cells and obtained ratio-images of intracellular [Ca^{2+}] under various conditions. We observed differences in Ca^{2+} movements between the cytoplasm and nucleus, which could be explained by assuming that the nuclear envelope restricts passive diffusion of cytoplasmic Ca^{2+} into the nucleus.

MATERIALS AND METHODS

Preparation of cardiac myocytes

Ventricular myocytes were isolated by a previously described procedure (19). Guinea pigs of either sex, weighing 300 to 400 g (Charles River, Yokohama), were anesthetized with pentobarbital sodium (40 to 50 mg/kg, i.p.). The aorta of isolated hearts was cannulated and perfused with Tyrode's solution of the following composition: 143 mM NaCl, 4 mM KCl, 0.5 mM $MgCl_2$, 1.8 mM $CaCl_2$, 0.33 mM NaH_2PO_4, 5.5 mM glucose and 5 mM HEPES. After the blood was washed out, the heart was perfused successively with nominally Ca^{2+}-free Tyrode's solution for 10 min and the same solution containing 0.6 mg/ml collagenase (Yakult, Tokyo) for about 10 min. All solutions were gassed with 100% O_2 and warmed to 36℃. Thereafter, the collagenase was washed out by 100 ml of the KB solution, i.e., "storage solution" described by Isenberg and Klockner (20) of the following composition: 70 mM glutamic acid, 15 mM taurine, 30 mM KCl, 10 mM KH_2PO_4, 0.5 mM $MgCl_2$, 11 mM glucose, 10 mM HEPES and 0.5 mM EGTA. The ventricle was then dissociated into single cells by gentle pipetting and stored in the KB solution at 4℃ until use.

Measurement of fluorescence intensity

Cells were placed in a chamber on the stage of the inverted microscope and perfused with the Tyrode's solution described above. When the effects of low [Na^+] were examined, the concentration of NaCl in the Tyrode's solution was reduced to 45 mM and instead, 90 mM choline chloride and 10^{-6} M atropine were added. Current and voltage clamp experiments were performed under the whole-cell configuration with a patch clamp amplifier (PC-one; Dagan, Minneapolis, MN, USA) and stimulator (SEN-3301; Nihon Kohden, Tokyo). The electrodes had a resistance of 2 to 4 MΩ and contained the following solution: 130 mM KCl, 5 mM HEPES, 1 mM $MgCl_2$, 5 mM K_2ATP and 0.2 mM indo-1 (pH 7.2). Action potentials were elicited at a frequency of 0.5 Hz under the current clamp mode with current pulses of 5-msec duration. In the voltage clamp experiments, a 3-sec depolarizing pulse to 0 mV was applied from a holding potential of −80 mV. The cells were excited at 351 nm from a high-power Ar^+ laser, and the emission bands, at 400−440 nm and more than 440 nm, were separated by dichroic mirrors and long pass filters, detected by parallel photo multipliers, and ratioed after correction of background fluorescence and shading. Full-field ratio-images were obtained at the rate of 15 frames/sec. The objective used was Nikon Fluor 40x/1.15 NA (water immersion). The fluorescence values were averaged over spots of 2 to 4 μm in diameter.

Calibration of intracellular [Ca^{2-}] from ratio values

As the ratio values of indo-1 were higher under the intracellular condition than in cell-free solutions (15), calibration was performed *in situ*. After loading the cell with indo-1 through patch pipettes, the extracellular solution was changed to the Ca^{2+}-free Tyrode's solution containing 10 mM EGTA, 5 μM FCCP, 5 μM rotenone and 10 μM ionomycin. After 10 to 20 min, the fluorescence ratio reached minimum values (R_{min}). Then the extracellular solution was changed to normal Tyrode's solution containing 5 μM FCCP, 5 μM rotenone and 10 μM ionomycin. After 1 to 2 min, the fluorescence ratio reached maximum values (R_{max}). Intracellular [Ca^{2+}] was calculated from the R_{min} and R_{max} values, and the K_d value of indo-1 (0.25 μM) according to the equation in the paper of Grynkiewicz et al. (21). The R_{min}, R_{max} and K_d values of indo-1 might be altered under the intracellular condition, which might result in calibration errors. Also, cell contraction occurred during the measurement of R_{max} values. Therefore, the results of the present study were presented in the figures both as ratio values and [Ca^{2-}].

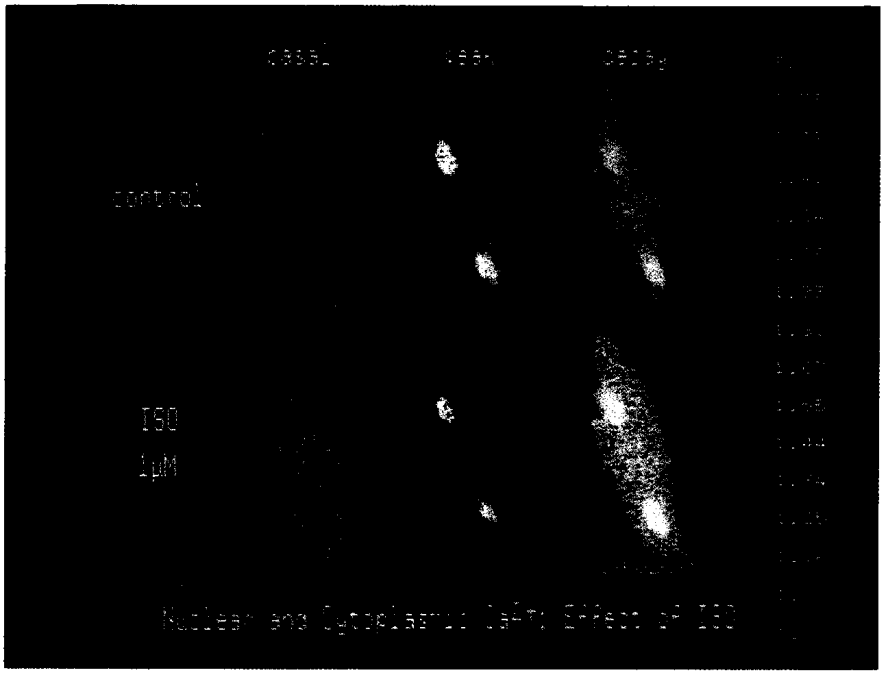

Fig. 1. Fluorescence ratio-images of [Ca^{2+}] in a cardiomyocyte. A guinea pig ventricular cardiomyocyte was loaded with indo-1 and stimulated at 0.5 Hz through a patch pipette. Ratio-images of indo-1 fluorescence were obtained at rest (just before stimulation: left panels), at the peak of the cytoplasmic Ca^{2-} transient (200 msec after stimulation: middle panels) and during decay (1 sec after stimulation: right panels) in the absence (upper panels) and presence (lower panels) of 1 μM isoproterenol. Each of the images was obtained in 67 msec. The color scale indicates fluorescence ratios. Two regions with smaller peak [Ca^{2+}] corresponds to the cell nuclei.

Drugs and chemicals

Indo-1 was purchased from Dojindo Laboratories (Kumamoto), collagenase from Yakult and isoproterenol from Sigma (St. Louis, MO, USA). All other chemicals are commercial products of the highest available grade of quality.

Statistics

Ratio and time values are expressed as means ±S.E.M. Statistical significance was evaluated by the paired *t*-test, and P values less than 0.05 were considered to indicate significant differences.

RESULTS

The two-dimensional image of Ca^{2+} movements in stimulated myocardial cells obtained every 67 msec could be distinguished from the preceding image and cytoplasmic and nuclear [Ca^{2+}] could be quantified. No difference in basal [Ca^{2+}] was observed between the cytoplasm and nucleus (Figs. 1–3). On stimulation, nuclear [Ca^{2+}] was observed to rise and fall following cytoplasmic [Ca^{2+}] with an obvious delay (Table 1), and the peak [Ca^{2+}] level was higher in the cytoplasm than in the nucleus (Figs. 1–3). No significant variations in [Ca^{2+}] was observed within the cytoplasm. Isoproterenol (1 μM) produced no change in the basal [Ca^{2+}] both in the cytoplasm and nucleus, but produced increases in the amplitude of the Ca^{2+} transient in both regions; the increase was larger in the cytoplasm than in the nucleus (Figs. 1–3). In most of the cells, the decrease in nuclear [Ca^{2+}] started before the cytoplasmic [Ca^{2+}] became lower than nuclear [Ca^{2-}] both in the absence and presence of isoproterenol (Fig. 2).

Under low [Na^{+}] condition (Figs. 4 and 5), the basal [Ca^{2+}] was elevated both in the cytoplasm and nucleus. In some cases, Ca^{2+} waves, a propagating local rise in intracellular [Ca^{2+}], was observed (for example, the cell

Table 1. Difference in time course of Ca^{2+} transients between the cytoplasm and nucleus

	Cytoplasm	Nucleus
Time to peak [Ca^{2+}]: msec	106.7±14.7	206.7±14.7*
Time for half decay ($t_{1/2}$): msec	196.7±10.5	293.3±12.0*

Time to peak [Ca^{2-}] and time for half decay ($t_{1/2}$) of [Ca^{2+}] were compared between the cytoplasm and nucleus. Data are presented as means±S.E.M. from 10 cells. * indicates a significant difference ($P<0.05$) from cytoplasmic values evaluated by the *t*-test.

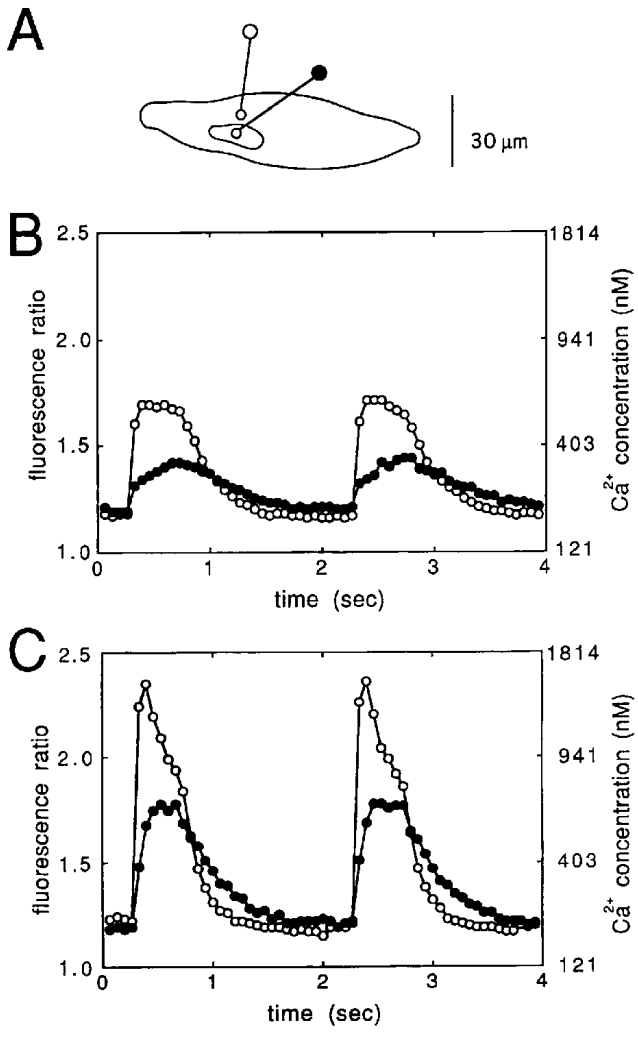

Fig. 2. Changes in fluorescence intensity of cytoplasmic and nuclear regions in the cell shown in Fig. 1. Fluorescence intensity of cytoplasmic (open circle) and nuclear (closed circle) regions as indicated in A was quantified every 67 msec in the absence (B) and presence (C) of 1 μM isoproterenol. $[Ca^{2+}]$ was expressed both as ratio values (left scale) and absolute concentration (right scale). No difference in basal $[Ca^{2+}]$ was observed between the cytoplasm and nucleus, but the peak and time course were different between the two regions.

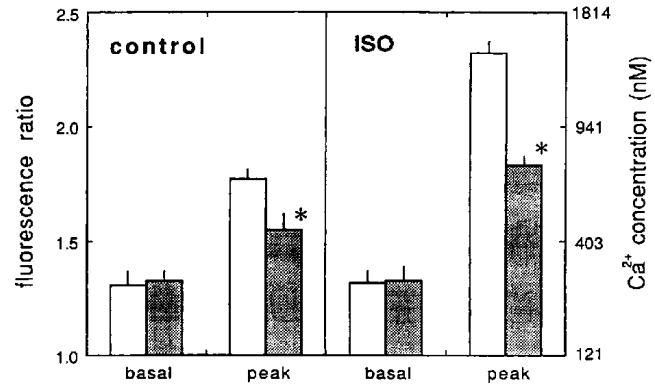

Fig. 3. Basal and peak $[Ca^{2+}]$ in the cytoplasm and nucleus. The same experiment as that shown in Fig. 2 was performed with 5 cells, and the basal and peak $[Ca^{2+}]$ in the cytoplasm (open columns) and nucleus (shadowed columns) before (control) and after application of 1 μM isoproterenol (ISO) were averaged. Columns with vertical bars indicate means $\pm$ S.E.M. of ratio values (left scale). Corresponding absolute values of $[Ca^{2+}]$ are indicated on the right scale. Asterisks on nuclear values indicate a significant difference ($P < 0.05$) from the corresponding cytoplasmic values.

shown in Fig. 7). Even under such a condition, no difference was observed in basal $[Ca^{2+}]$ between the cytoplasmic and nuclear regions. Low $[Na^{+}]$ also produced an increase in the amplitude of the Ca^{2+} transient both in the cytoplasm and nucleus; The increase was larger in the cytoplasm than in the nucleus (Figs. 4 and 5). In most of the cells, the decrease in nuclear $[Ca^{2+}]$ started before the cytoplasmic $[Ca^{2+}]$ became lower than the nuclear $[Ca^{2+}]$ under both normal and low $[Na^{+}]$ conditions (Fig. 4).

Artificial changes in cytoplasmic $[Ca^{2+}]$ was produced by voltage clamping the cell to observe the difference in the pattern of $[Ca^{2+}]$ changes between the cytoplasm and nucleus (Fig. 6). On depolarization to 0 mV from a holding potential of -80 mV, cytoplasmic $[Ca^{2+}]$ rapidly increased to reach a peak at 133 msec after depolarization, followed by a decrease, and was then maintained at constant level as long as the membrane was clamped at 0 mV. In contrast, no peak was observed in nuclear $[Ca^{2+}]$, which slowly increased to reach the cytoplasmic level after 533 msec and remained constant thereafter. On repolarization, cytoplasmic $[Ca^{2+}]$ rapidly changed to reach a decreased level in 200 msec, while nuclear $[Ca^{2+}]$ decreased to the same level more slowly.

In some cells, "Ca^{2+} waves", a slowly propagating local increase in $[Ca^{2+}]$, were observed under the low $[Na^{+}]$ condition. In the cell shown in Fig. 7, a wave emanated from a site close to the nucleus and propagated around the nucleus to reach the opposite side. Quantification of $[Ca^{2+}]$ was performed at four different sites in the cell (Fig. 7): A is the wave initiation site, B and C are in the cytoplasm around the nucleus, and N is inside the nucleus. B and C are at equal distance from A of about 20 μm, but the nucleus lies between A and C. The wave propagated around the nucleus from A to B and from B to C with a velocity of 100 μm/sec. The nuclear $[Ca^{2+}]$ (N) also increased but was slow in time course and small in amplitude when compared with that of the cytoplasm.

DISCUSSION

Several recent papers using confocal microscopy and Ca^{2+}-sensitive fluoroprobes have demonstrated a differ-

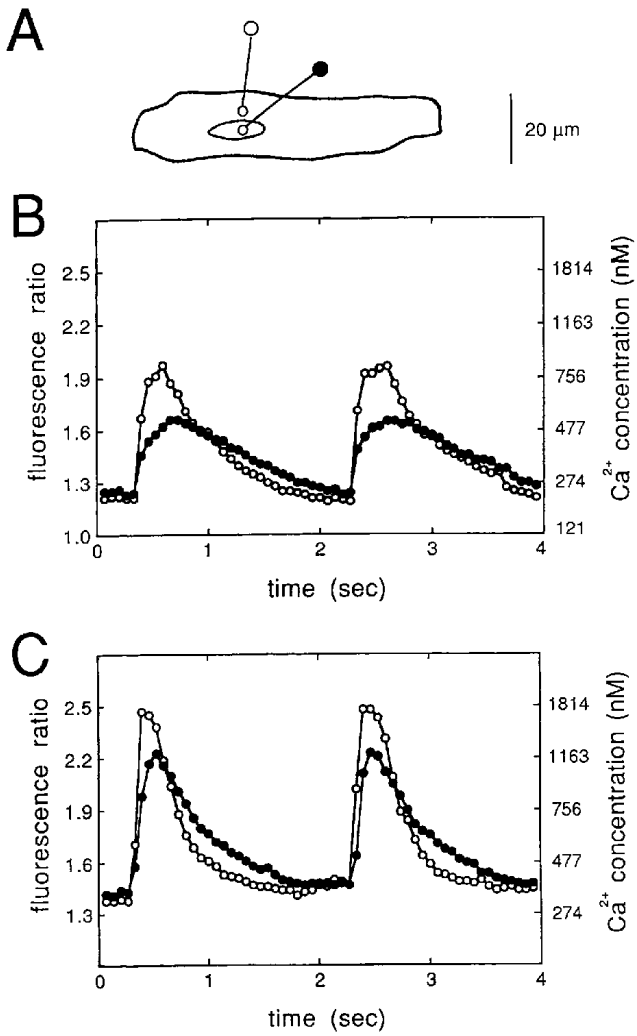

Fig. 4. Changes in fluorescence intensity of cytoplasmic and nuclear regions under the normal and low [Na^+] condition. Fluorescence intensity of cytoplasmic (open circle) and nuclear (closed circle) regions as indicated in A was quantified every 67 msec under the normal (B) and low [Na^+] condition (C). [Ca^{2+}] was expressed both as ratio values (left scale) and absolute concentration (right scale). The basal [Ca^{2+}] was elevated under the low [Na^+] condition, but no difference in basal [Ca^{2+}] was observed between the cytoplasm and nucleus.

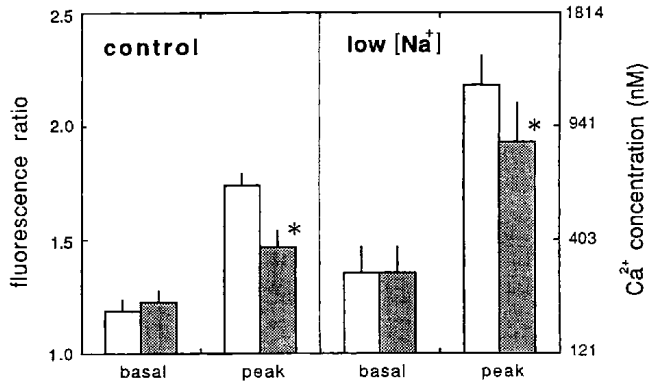

Fig. 5. Basal and peak [Ca^{2+}] in the cytoplasm and nucleus. The same experiment as that shown in Fig. 4 was performed with 5 cells and the basal and peak [Ca^{2+}] in the cytoplasm (open columns) and nucleus (shadowed columns) under normal (control) and low [Na^+] condition were averaged. Columns with vertical bars indicate means±S.E.M. of ratio values (left scale). Corresponding absolute values of [Ca^{2+}] are indicated on the right scale. Asterisks on nuclear values indicate a significant difference ($P<0.05$) from the corresponding cytoplasmic values.

ence in [Ca^{2+}] between the cell nucleus and the cytoplasm. In the cultured rat hepatocyte, nuclear [Ca^{2+}] was lower than that of the cytoplasm under basal conditions, but after stimulation with phenylephrine, the [Ca^{2+}] of the two components increased to a similar level (15). In B cells (5) and mast cells (6), nuclear [Ca^{2+}] was reported to become higher than that of the cytoplasm after stimulation of the cells with an antigen and compound 48/80, respectively. On the other hand, no difference in nuclear and cytoplasmic [Ca^{2+}] was observed in basophilic leukemia cells (10). A report on neuroblastoma demonstrated that the difference in nuclear and cytoplasmic Ca^{2+} movements can be explained by the nuclear envelope serving to restrict passive diffusion of cytoplasmic Ca^{2+} into the nucleus (9). The authors raised the possibility that the amplification of Ca^{2+} signals in the nucleus observed in neurons may be produced by measurement artifacts. Brini et al. (22) used nucleoplasm directed aequorin and demonstrated no difference in cytoplasmic and nuclear [Ca^{2+}]. Thus, whether nuclear [Ca^{2+}] is regulated independently of cytoplasmic [Ca^{2+}] is still controversial.

In the case of myocardial cells, we have observed in cultured ventricular cells that nuclear [Ca^{2+}] rises and falls following cytoplasmic [Ca^{2+}] with an obvious delay (18). This is in agreement with a recent report on cultured cardiomyocytes (14). These observations suggest that some regulatory mechanisms may exist for nuclear [Ca^{2+}]. However, as fluo-3 was used in our previous study, it was difficult to compare the [Ca^{2+}] between the two regions. Thus, in the present study, we used the ratiometric fluoroprobe, indo-1, which enables us to directly compare the [Ca^{2+}] between intracellular regions. Errors introduced by regional differences in fluoroprobe concentration are corrected by calculating the ratio of the fluorescence intensities at two emission wavelengths, and intracellular [Ca^{2+}] can be calibrated from the ratio values. In our preliminary experiments, we applied the acetoxymethyl ester of indo-1 (indo-1/AM) to the myocytes and obtained ratio-images under field stimulation. However, there seemed to be considerable amount of dye compartmentalization into cytoplasmic organelles, especially when the loading time and/or loading concentra-

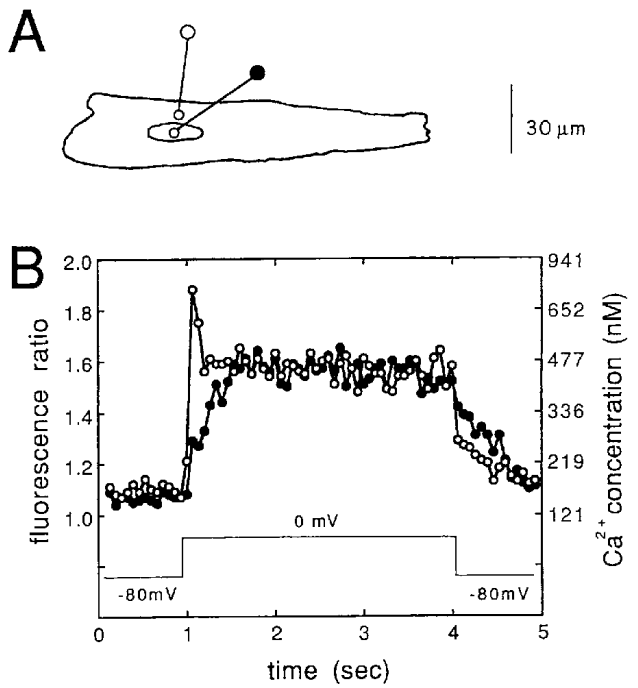

Fig. 6. Cytoplasmic and nuclear $[Ca^{2+}]$ under voltage clamp. Fluorescence intensity of cytoplasmic (open circle) and nuclear (closed circle) regions as indicated in A was quantified every 67 msec, and the time course is shown in B. A depolarizing command to 0 mV for 3 sec from a holding potential of −80 mV was applied as indicated. Cytoplasmic $[Ca^{2+}]$ rapidly increased to reach a peak at 133 msec after depolarization, followed by a decrease, and was then maintained at a constant level as long as the membrane was clamped at 0 mV. Nuclear $[Ca^{2+}]$ slowly increased to reach the cytoplasmic level and remained constant thereafter. Decrease in $[Ca^{2+}]$ on repolarization was also slower in the nucleus.

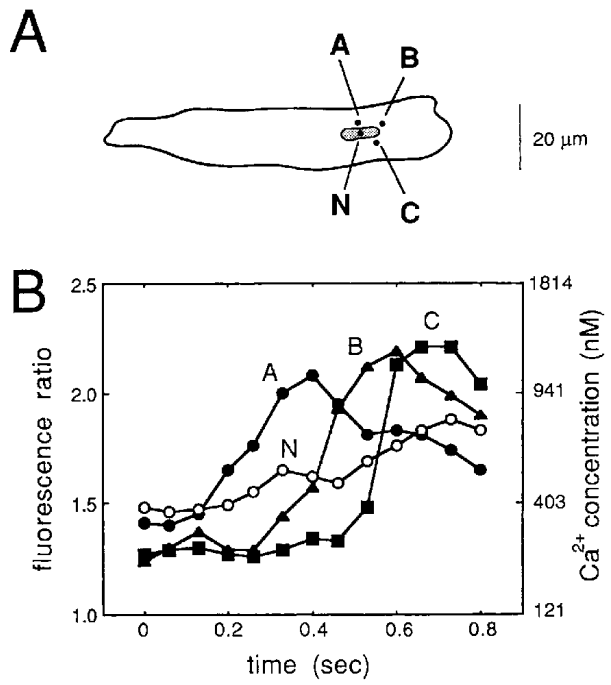

Fig. 7. A Ca^{2+} wave propagating around a nucleus. In the cell shown in A, a "Ca^{2+} wave" a slowly propagating local increase in $[Ca^{2+}]$, were observed under the low $[Na^{+}]$ condition. Quantification of $[Ca^{2+}]$ was performed at four different sites in the cell: A (closed circles) is the wave initiation site, B (triangles) and C (squares) are in the cytoplasm around the nucleus (shadowed region), and N (open circles) is inside the nucleus. B and C are at equal distance from A of about 20 μm, but the nucleus lies between A and C. The wave propagated from A to B and from B to C with a velocity of 100 μm/sec. The nuclear $[Ca^{2+}]$ (N) also increased but was slow in time course and small in amplitude when compared with that of the cytoplasm.

tion was increased. The distribution of residual fluorescence after solublization of the cell membrane with digitonin showed a close correlation with the staining pattern by rhodamine 123, which suggested that a considerable amount of indo-1 might have been taken up into mitochondria. Such accumulation of dye in intracellular organelles has been reported to occur during acetoxymethylester loading, which leads to various artifacts such as apparent nuclear amplification of Ca^{2+} signals (23). Therefore, we decided to introduce free indo-1 into the cytoplasm through patch pipettes; this method is considered to be less likely to cause such errors.

The basal $[Ca^{2+}]$ level was not different between the nucleus and cytoplasm under the control condition, after ISO treatment and after elevation by low $[Na^{+}]$ treatment (Figs. 1–5). On stimulation, cytoplasmic $[Ca^{2+}]$ increased rapidly to reach a peak after about 133 msec, followed by a slower and smaller increase in nuclear $[Ca^{2+}]$ (Figs. 2–5). These results can be explained by assuming that the nuclear envelope restricts Ca^{2-} diffusion from the cytoplasm to the nucleoplasm. Minamikawa et al. (14) have reported that the basal $[Ca^{2+}]$ of the nucleus was lower than that of the cytoplasm; the reason for this discrepancy is not clear at present, but the difference in experimental conditions might be involved. For example, Minamikawa et al. used cultured myocytes and acetoxymethyl esters of fluoroprobes. We further forced cytoplasmic $[Ca^{2+}]$ to change in an artificial pattern by voltage clamping. When the membrane potential of a ventricular myocyte was clamped at 0 mV, cytoplasmic $[Ca^{2+}]$ was maintained at a constant level after the initial peak (Fig. 6), which can be explained by persistent Ca^{2+} influx through the "calcium current window" and inhibition of transsarcolemmal Ca^{2+} extrusion via the Na^{+}-Ca^{2+} exchange under such a condition. Nuclear $[Ca^{2+}]$ could not follow the initial rapid rise in cytoplasmic $[Ca^{2+}]$, but the steady state $[Ca^{2+}]$ level during depolarization was the same as that of the cytoplasm. Thus, it was confirmed that nuclear $[Ca^{2+}]$ follows cytoplasmic $[Ca^{2+}]$ with a delay.

Ca^{2+} waves, slowly propagating local increases in intracellular [Ca^{2+}], has been observed in many cell types, including myocardial cells. The propagation velocity of the Ca^{2+} wave shown in Fig. 7 was 100 μm/sec, which agrees with previous observations of waves in myocardial cells (13, 18, 24, 25). Ca^{2+} waves in myocardial cells are considered to be due to localized activation of the Ca^{2+}-induced Ca^{2-}-release mechanism (13, 18, 24, 25). In the present study, propagation of waves were slowed by the presence of the nucleus (Fig. 7), which was in agreement with previous observations (12, 14, 26). In some cases, waves were observed to propagate repeatedly around the nucleus. Thus, the wave of Ca^{2+}-induced Ca^{2+}-release does not seem to propagate into the nucleoplasm, at least not with a velocity and amplitude comparable to that in the cytoplasm.

Most of our present results could be explained by assuming that nuclear Ca^{2+} originates from cytoplasmic Ca^{2+}, the nuclear envelope simply restricting passive diffusion of cytoplasmic Ca^{2+} into the nucleus. However, there is one point that is difficult to explain by this model: the decrease in nuclear [Ca^{2-}] starts before the cytoplasmic [Ca^{2+}] becomes lower than nuclear [Ca^{2+}] (Figs. 2 and 4). This observation leads to the speculation that factors independent of cytoplasmic [Ca^{2+}], such as membrane repolarization, might somehow trigger or modify the decrease in nuclear [Ca^{2+}]. Some investigators postulate that in non-myocardial cells, nuclear Ca^{2+} may originate from a pool distinct from that for cytoplasmic Ca^{2-} such as the nuclear envelope or some intranuclear store site not identified at present (3, 8). This possibility can not be ruled out from the present results. The nucleus was reported to posses Ca^{2+} pump activity that is responsible for the accumulation of Ca^{2+} in the nucleus (27). None of our present results can rule out the possibility that nuclear Ca^{2+} originates from a non-cytoplasmic store site. Thus, although our present results from myocardial cells can be best explained by passive diffusion of cytoplasmic Ca^{2+} into the nucleus at present, definitive conclusions await further investigation.

Some reports postulate that nuclear Ca^{2+} may be involved in intranuclear events such as gene expression (28), breakdown of nucleic acids and apoptosis (29). The presence in the nucleus of calmodulin and Ca^{2+}-sensitive enzymes have been reported in various cell types (30–33). Ca^{2+} was also reported to be involved in phosphorylation of non-histone proteins in the nucleus (34). In vascular smooth muscle cells, stimulation by angiotensin II results in elevation of both cytoplasmic and nuclear [Ca^{2+}] and induction of c-*myc* and c-*fos* expression, which is considered to play a role in cell proliferation (35, 36). Some investigators propose that, in neuronal cells, cytoplasmic and nuclear enzymes may be differentially activated depending on the excitation pattern of the cell (9). Our present data on myocardial cells demonstrated that increase in basal and peak [Ca^{2+}] by endogenous transmitters, as well as a local increase in cytoplasmic [Ca^{2+}], are reflected in nuclear [Ca^{2+}]. Thus, it is possible that changes in the excitation pattern of myocardial cells either by endogenous transmitters or under pathophysiological conditions such as arrhythmia or hypoxia may influence their intranuclear events through changes in nuclear Ca^{2+} kinetics.

Acknowledgments

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