Expression and Function of Ryanodine Receptors in Nonexcitable Cells*

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We have used reverse transcriptase-polymerase chain reaction to investigate the expression of ryanodine receptors in several excitable and nonexcitable cell types. Consistent with previous reports, we detected ryanodine receptor expression in brain, heart, and skeletal muscle. In addition, we detected ryanodine receptor expression in various other excitable cells including PC12 and A7r5 cells. Several muscle cell lines (BC3H1, C2C12, L6, and Sol8) weakly expressed ryanodine receptor when undifferentiated but strongly expressed type 1 and type 3 ryanodine receptor isoforms when differentiated into a muscle phenotype. Only 2 (HeLa and LLC-PK1 cells) out of 11 nonexcitable cell types examined expressed ryanodine receptors. Expression of ryanodine receptors at the protein level in these cells was confirmed using [3H]ryanodine binding. We also investigated the function of ryanodine receptors in Ca2+ signaling in HeLa cells using single-cell Fura-2 imaging. Neither caffeine nor ryanodine caused a detectable elevation of cytoplasmic Ca2+ in single HeLa cells. However, ryanodine caused a significant decrease in the amplitude of Ca2+ signals evoked by repetitive stimulation with ATP. These studies show that ryanodine receptors are expressed in some nonexcitable cell types and furthermore suggest that the ryanodine receptors may be involved in a subtle regulation of intracellular Ca2+ responses.

Cells have two major mechanisms available for regulating the release of internal Ca2+. In one case, external signals acting on receptors at the cell periphery generate the second messenger inositol 1,4,5-trisphosphate, which diffuses into the cell and mobilizes Ca2+ by engaging inositol 1,4,5-trisphosphate receptors on the endoplasmic reticulum (1). The other mechanism employs a related, but distinct, family of intracellular channels, the ryanodine receptors (RyRs), so called because they strongly bind the plant alkaloid ryanodine (2). RyRs are expressed in various tissues using RT-PCR. In addition to finding RyRs in several excitable tissues and cell lines, we detected RyR expression in various nonexcitable tissues and cell lines. For example, effects of ryanodine on agonist-induced calcium signals in intact cells (9, 10) and specific ryanodine binding sites in hepatocyte vesicles (11, 12) have been demonstrated. However, the pharmacology of the putative RyR in hepatocytes is very different from those expressed in muscle tissues (9, 13), and furthermore, the expression of RyR mRNA in hepatocytes has not been detected using molecular techniques (7, 11, 14, 15).

For other nonexcitable cells the converse problem exists, in that molecular techniques have identified RyR mRNA expression, but the functional evidence has been confusing, since some RyR-activating agents fail to evoke responses in these cells. For example, in mammalian epithelial cells and Jurkat T-lymphocytes, which appear to express RyR3, effects of ryanodine but not caffeine have been observed (3, 16). In a separate study Guse et al. (17) found that Jurkat cells were caffeine-responsive.

In the present study we have examined RyR expression in a variety of tissues using RT-PCR. In addition to finding RyRs expressed in various excitable tissues and cell lines, we detected RyR expression in two nonexcitable cell types. For one of these nonexcitable cell types (HeLa cells), we investigated the function of the expressed RyRs using single-cell Fura-2 imaging.

MATERIALS AND METHODS

CDs—A7r5 embryonic rat aorta cells, RBL-2H3 rat mucosal mast cells, GH3 rat pituitary tumor cells, ChH1 10T1/2, and Swiss 3T3 embryonic mouse fibroblasts, L cells from mouse connective tissue, BC3H1 mouse embryonic myoblasts, Rin m5F rat insulinoma cells, and PC12 pheochromocytooma cells were obtained and cultured as described by De Smedt et al. (18), Jurkat T cell leukemia cells as described by Parys et al. (19), HeLa carcinoma cells as described by Bootman et al. (20), and LLC-PK1 renal epithelial cells as described by Parys et al. (21). L6 cells and C2C12 cells were obtained from the ATCC (CRL 1458 and CRL 1772, respectively) and cultured as described by Florini and Magri (22). C6 cells were obtained from the ATCC (CCL 107) and cultured using the recommended conditions. Sol8 cells were a gift from Dr. C. Pinset (Institut Pasteur, Centre National de la Recherche Scientifique, Paris, France), and HUVEC cells freshly isolated from human umbilical vein...
were provided by Dr. D. Colen (Centre for Molecular and Vascular Biology, KU Leuven, Belgium). PC12 cells were differentiated as described previously (23). Sol8 and BC3H1 cells were differentiated by reducing the concentration of fetal calf serum in the growing medium to 0.5%. For differentiation of C2C12 cells, cells were grown in medium containing 1% horse serum and 0.5% insulin transferrin selenite. Differentiation was evoked by each wavelength of 750–580 nm (24). For single-cell imaging of HeLa cells, cells were transferred from plastic culture dishes to glass coverslips (22 mm diameter, Chance Propeller Ltd, Smethwick, Warley, UK). Cells were allowed to attach to the coverslips for 4 h before use.

Reverse Transcription and PCR Amplification—Total RNA was prepared from 1.6 × 10^6 cells and cell homogenates as described previously (19). Sol8 and BC3H1 cells were differentiated by reducing the concentration of fetal calf serum in the growing medium to 0.5%. For differentiation of C2C12 cells, cells were grown in medium containing 1% horse serum and 0.5% insulin transferrin selenite. Differentiation was evoked by each wavelength of 750–580 nm (24). Random primed first strand cDNA was synthesized from 1 μg of RNA using avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim). RyR cDNAs were amplified using degenerate primers based on sequences conserved among the three RyR isoforms (rabbit RyR1, RyR2, RyR3; human RyR1, RyR3; pig RyR1) and corresponding to the putative membrane-spanning regions M3 and M4 (25). The sequences of the primers and their position within the rabbit RyR sequences are shown below.

Forward primer (RyR1, 14419–14441; RyR2, 14230–14252; RyR3, 14008–14030) was as follows: 5′-CA(A/G)T(C/T)(A/G)T(A/G)T(C/T)(A/G)C(A/G/C/T)GA(C/T)ATTA(C/G/T)CT/TC-3′. Reverse primer (RyR1, 14929–14951; RyR2, 14740–14762; RyR3, 14518–14540) was as follows: 5′-AAC(A/G)T(A/G)T(A/G)T(C/T)(A/G)C(A/G/C/T)GA(C/T)ATTA(C/G/T)CT-TC-3′. PCR reactions (100 μl) contained 0.2–2% of the first strand cDNA template, 100 pmol of each primer, 0.2 mM dNTPs and 2.5 units of Taq polymerase (Boehringer Mannheim) in 10 mM Tris/HCl (pH 8.3), 50 mM KCl, and 1.5 mM MgCl2. Reactions were carried out in a Biometra UNO-Thermoblock for 30 cycles using a denaturing step for 1 min at 94°C, annealing for 1 min at 50°C, and extension for 1 min at 72°C. This was followed by a final extension step at 72°C for 10 min.

Cloning and Sequencing of RT-PCR Products—PCR products were fractionated on a 1.5% agarose gel, and individual bands were purified using the Geneden II DNA purification kit (Bio 101, Inc.). The purified DNA was cloned into the plasmid vector pGEM-T (Promega) according to the manufacturer’s instructions, and clones containing inserts of the expected size were sequenced in both directions by dideoxy sequencing using the Sequenase version 2.0 kit (Amersham Corp.).

Restriction Enzyme Analysis of RT-PCR Products—RT-PCR products were subjected to restriction enzyme analysis to determine which isoforms were present. For mouse RT-PCR products, three restriction enzymes, Sad, FokI, and Aval, were chosen. Sad was predicted to cut RyR1 and RyR2 into fragments of approximately 395 and 135 base pairs (bp). FokI was predicted to cut RyR1 into 500- and 30-bp fragments and cut RyR2 into 200-, 180-, and 150-bp fragments. Aval was expected to cut RyR1 into 270- and 260-bp fragments and RyR2 into 400- and 130-bp fragments. For rat RT-PCR products, the restriction enzymes Sad, BgiII, and Ead were chosen. Sad was predicted to cut RyR1 and RyR2 into fragments (EaeI was expected to cut RyR3 only into 280- and 250-bp fragments). BgiII should cut RyR3 only into 270- and 260-bp fragments, and Ead should cut RyR3 only into 350- and 180-bp fragments. Human and pig RT-PCR products, the restriction enzymes Sad, BgiII, and BseEI were chosen. Sad was expected to cut RyR1 only into 300- and 130-bp fragments, BgiII was expected to cut RyR2 only into 295- and 235-bp fragments, and BseEI was expected to cut RyR3 only into 280- and 250-bp fragments.

Microsomal Preparation and ([3H]Ry)yanodine Binding—Total microsomes were isolated from the various cell lines and from whole rat brain as described previously (19). The binding assay was based on the optimized conditions described for ryanodine binding to the rat brain ryanodine receptor (26). Binding to microsomes (1.25 mg/ml) was measured in a mixture containing 100 mM NaOH (pH 7.4), 2 mM ATP, 0.89 mM MgCl2, 1.8 mM CaCl2, 6 mM NaHCO3, 5.5 mM glucose, 25 mM Hepes (pH 7.3). Cells were loaded with 2 μM Fura-2-aceatoxymester (Molecular Probes Inc.) by incubation for 35–40 min at room temperature (20°C) followed by an extracellular medium wash and a further 20-min incubation to allow for absolute control of the loaded dye. Coverslips were mounted at room temperature on the stage of a Nikon diaphot inverted epifluorescence microscope. Fluorescent images were obtained at video rate (40 ms exposure time) by alternate excitation at 340 or 380 nm using an image-processing system (Imagine, Synoptics Ltd, Cambridge, UK) interfaced to a DEC MicroVAX II microcomputer and then filtered with a 200-ms time constant. The emission signal at 510 nm was collected by a charge-coupled device intensifying camera (Photonic Science, Robertsbridge, Kent, UK), and the digitized signals were stored and processed as described previously (28). The responses shown in the present study were obtained from four independent trials for each experiment.

RESULTS

RT-PCR Amplification of RyR Isoforms—To search for RyRs in a range of excitable and nonexcitable cell types from different species we used a RT-PCR assay. In this technique, RNA is used as a template for an initial reverse transcription step to produce cDNA, followed by amplification of specific sequences using PCR. By aligning the previously published RyR sequences (see "Materials and Methods"), we designed degenerate PCR primers to amplify an approximately 530-bp product from the 3′ region of each RyR isoform. This region is highly conserved between isoforms and species but, importantly, also contains sufficient differences to allow the three RyR isoforms to be distinguished. The intron-exon structure of this region has been determined for two RyR isoforms. In the case of pig RyR1 genomic DNA, the primers would be expected to amplify a fragment that spans 4 introns (approximately 1700 bp), and within human RyR3 genomic DNA, the primers should amplify a fragment that spans at least 1 intron. Interestingly, the intron identified within the human RyR3 gene is in the same position as an intron in the pig RyR1 gene, suggesting that some intron splice sites may be conserved between RyR isoforms and animal species. Therefore, although the intron-exon structure of all three RyR isoforms from each species tested has not been determined, it seems unlikely that PCR amplification of any contaminating genomic DNA would produce the correctly sized PCR product (530 bp).

As the majority of cell types tested were of mouse, rat, or human origin, and all three RyR isoforms have been found in various regions of the brain (7, 31), we tested the primers by carrying out PCR amplification of cDNA from mouse, rat, and human brain. A product of the predicted size (530 bp) was amplified from all three brain cDNAs (Fig. 1A). Control reactions containing H2O or RNA were negative in the PCR (Fig. 1A). Cloning and sequencing the brain PCR products revealed that the primers were able to specifically amplify all three RyR isoforms. The primers were also able to specifically amplify RyRs from mouse heart (RyR2 and RyR3) and skeletal muscle (RyR1 and RyR3) (data not shown).

A range of cell types was subjected to PCR amplification using these primers (Fig. 1B). Only two of the nonexcitable cell types tested, HeLa and LLC-PK1 cells, yielded detectable PCR products. The other nonexcitable cell types, including fibroblasts and J urkat cells, appeared negative in the PCR. As a positive control for the quality of the cDNA, amplification reactions were also carried out using β-actin PCR products. Products of the correct size and of equivalent intensity were ampli-
fied from all cDNAs tested in this study (data not shown).

Using the RyR primers, products of the predicted size were detected in most excitable cell types, including differentiated and undifferentiated PC12 cells and A7r5 cells. A very faint PCR product was detected in GH3 and Rin m5F cells, indicating that these cell types probably also express RyRs, but very weakly. An interesting pattern of expression was detected in the BC3H1, Sol8, C2C12, and L6 muscle cell lines. By adjusting the culture conditions appropriately (see "Materials and Methods"), these cells can either be maintained in a rapidly growing non-muscle state or differentiated into a muscle phenotype. In the undifferentiated cells, we detected only very weak expression of RyR by PCR. However, differentiation of the cells correlated with the appearance of much more abundant levels of the 530-bp product (Fig. 1B).

**Tissue-specific Distribution of RyR Isoforms—** The amplification products obtained from most of the PCR-positive tissues were cloned and sequenced to determine which isoforms were expressed. Although we sequenced between 5 and 20 clones obtained from these PCR-positive cell types, in some only one or two RyR isoforms were detected. To confirm that these were the only isoforms amplified, we performed restriction digests on these PCR products, using enzymes specific for each isoform (see "Materials and Methods").

Fig. 2 shows the restriction digest analysis of RyR PCR products amplified from differentiated BC3H1 cells, differentiated L6 cells, and HeLa cells. The BC3H1 PCR product was partially cut with SacI (RyR1-specific) and completely cut with Avall (digests RyR1 and -3) to produce fragments of the expected sizes, suggesting that BC3H1 cells express RyR1 and RyR3. The L6 PCR product was completely cut with SacI and not cut by either BglII (RyR2-specific) or EcoRI (RyR3-specific). The HeLa cell PCR product was completely cut with BglII.
(RyR2-specific) to produce fragments of the expected size. The combined results obtained from sequencing and restriction enzyme digest of the PCR products are presented in Table I.

\[ \text{Table I} \]

**Distribution of RyR isoforms in excitable and nonexcitable cells**

| Excitable cells | PCR | RyR1 | RyR2 | RyR3 |
|-----------------|-----|------|------|------|
| Skeletal muscle (mouse) | + | + | + | + |
| Heart (mouse) | + | + | + | + |
| Brain (mouse, rat, and human) | + | + | + | + |
| Mouse | | | | |
| BC3H1 (undifferentiated) myoblasts | + | + | + | + |
| BC3H1 (differentiated) myocytes | + | + | + | + |
| C2C12 (undifferentiated) myoblasts | + | + | + | + |
| C2C12 (differentiated) myocytes | + | + | + | + |
| Sol8 (undifferentiated) myoblasts | + | + | + | + |
| Sol8 (differentiated) myocytes | + | + | + | + |
| Rat | | | | |
| L6 (undifferentiated) myoblasts | + | + | + | + |
| L6 (differentiated) myocytes | + | + | + | + |
| PC12 (undifferentiated) pheochromocytoma | + | + | + | + |
| PC12 (differentiated) pheochromocytoma | + | + | + | + |
| A7r5 aorta | + | + | + | + |
| Rin m5F insulinoma | + | + | + | + |
| GH3 pituitary tumor | + | + | + | + |
| Nonexcitable cells | PCR | RyR1 | RyR2 | RyR3 |
|------------------|-----|------|------|------|
| Mouse | | | | |
| CH3 10T1/2 embryonic fibroblasts | - | - | - | - |
| Swiss 3T3 cells embryonic fibroblasts | - | - | - | - |
| L-cell fibroblasts | - | - | - | - |
| Rat | | | | |
| Liver | - | - | - | - |
| RBL-2H3 mast cells | - | - | - | - |
| C6 glial tumour | - | - | - | - |
| Human | | | | |
| Jurkat T cells | + | + | + | + |
| HeLa cervix carcinoma | + | + | + | + |
| HUVEC umbilical vein endothelial cells | + | + | + | + |
| K562 erythroleukaemia | + | + | + | + |
| Pig | | | | |
| LLC-PK1 kidney proximal tubule | + | + | + | + |

\[ ^a \text{Cell types that were positive in the PCR are indicated with a plus (+), and those that were negative with a minus (-).} \]

\[ ^b \text{The particular isoforms that were detected by sequencing/restriction digestion are indicated with a plus (+).} \]

\[ ^c \text{Specific isoforms expressed not determined.} \]

**Ryanodine Receptors in Nonexcitable Cells**

The finding that some nonexcitable cells expressed RyR mRNA was surprising, since RyRs were previously thought to be restricted to excitable tissues. In addition, there is conflicting evidence on the expression of ryanodin receptors in A7r5 cells (32, 33). We therefore sought to confirm the PCR results and to obtain evidence for RyR expression at the protein level, using a \(^3\)H ryanodine binding assay. The trace in A shows the response of a single Fura-2-loaded HeLa cell perfused with solutions containing increasing caffeine concentrations from 2 to 40 mM (filled bars). Histamine (100 \(\mu\)M; hatched bar), which releases \(Ca^{2+}\) from intracellular stores (20), was applied to show that the \(Ca^{2+}\) stores did contain releasable \(Ca^{2+}\) and to demonstrate the typical magnitude of hormonally evoked \(Ca^{2+}\) signals. The trace is typical of 40 cells. The trace in B shows the response of a single Fura-2-loaded HeLa cell perfused with solutions containing caffeine (10 mM; filled bar), ryanodine (10 \(\mu\)M; open bar), or caffeine (10 mM) + ryanodine (10 \(\mu\)M). Histamine (100 \(\mu\)M; hatched bar) was again added as a control. The trace is typical of 34 cells.
pression in Jurkat cells.

Attenuation of HeLa Cell Ca\textsuperscript{2+} Responses by Ryanodine—The PCR and restriction digest analysis (Fig. 2 and Table I) and \[^{3}\text{H}\]ryanodine binding data (Fig. 3) suggested that HeLa cells express RyR2. We investigated the potential contribution of these channels to intracellular calcium signals using single-cell imaging. Application of caffeine (2–40 mM) to the cells did not evoke any measurable \([\text{Ca}^{2+}]_i\) increase (Fig. 4A). In addition, treatment with ryanodine (10 mM) failed to give detectable changes in \([\text{Ca}^{2+}]_i\) when applied alone or in combination with 10 mM caffeine (Fig. 4B).

The lack of caffeine effect suggested that the HeLa cells either expressed a caffeine-insensitive RyR isoform or had a low RyR density. We therefore altered the experimental protocol to maximize the potential effect of the limited number of RyRs. HeLa cells were repetitively stimulated with ATP (100 \(\mu\)M), which mobilizes \(\text{Ca}^{2+}\) from intracellular stores in HeLa cells (35), either in the absence or presence of ryanodine (10 \(\mu\)M) (Fig. 5, A and B, respectively). The averaged \([\text{Ca}^{2+}]_i\) values for peaks 2–7 are shown in Fig. 5, C and D. In control cells, the magnitude of the response to repeated ATP applications decayed only slightly, probably due to desensitization of the \(\text{Ca}^{2+}\)-signaling pathway (35). The magnitude of ATP-evoked responses in the cells treated with ryanodine diminished significantly, so that the response decayed to about 50% after six ATP applications. The latency of the response to ATP slightly increased in the presence of ryanodine (Fig. 5E).

**DISCUSSION**

The aim of the present study was to investigate the possible expression and function of RyRs in nonexcitable cells. To confirm that our PCR technique could detect RyR expression, we also analyzed several tissues known to have functional RyRs. Consistent with other studies (7, 31), we could amplify and clone all three RyR isoforms from brain samples (Fig. 1A and Table I). In addition, we found RyR expression in skeletal and cardiac muscle. RyR3 appeared to be expressed in both these muscle types, whereas RyR1 and RyR2 were expressed in skeletal and cardiac muscle, respectively (Table I).

Of particular interest were the myogenic cell lines BC3H1, SOL8, C2C12, and L6, where the intensity of the 530-bp PCR product correlated with the differentiation of the cells from a non-muscle to a muscle phenotype. Although the PCR method we used was not quantitative, for a limited number of PCR cycles the intensity of the 530-bp PCR product may give an indication of the level of RyR mRNA expression. Our observations are consistent with several other studies linking muscle differentiation with RyR expression (7, 23, 36). It appears that
the myogenic cells express RyR1 (L6) or RyR1 and RyR3 (BC3H1, Sol8, and C2C12) after differentiation, similar to the situation in skeletal muscle (7) (Table I). Both undifferentiated and nerve growth factor-differentiated PC12 cells were RyR-positive (Table I). However, there was no apparent change in isoform expression pattern from RyR1 and RyR2 in undifferentiated cells to only RyR1 in differentiated cells. The expression of RyR1 in undifferentiated PC12 cells may explain the observation that thephyllyline is equipotent with caffeine at stimulating Ca^{2+} release from this clone (34), since RyR1 appears equally sensitive to these two methylxanthines (37).

Several previous studies have suggested that RyR expression is growth state-dependent; for example, aortic smooth muscle cells lose caffeine sensitivity during their logarithmic proliferation phase, which can be reversed or augmented by the removal or addition of growth factors, respectively (38). Similarly, the expression of RyR3 in mink lung epithelial cells can be induced by exposure to TGF-β (3). These data suggest that RyR expression can be somewhat labile and may be associated with the state of cell proliferation and differentiation.

After confirming that we could detect RyR expression in these excitable tissues, we investigated the potential expression of these receptors in 11 nonexcitable cell types, many of which are commonly used for studies of Ca^{2+} signaling (Table I). Of these cell types, only two appeared to express RyR mRNA. Increasing the number of PCR cycles or performing sequential PCR reactions, using the first reaction to prime the second, did not yield detectable products for the cell types that were RyR-negative. The group of cell types that were found not to express RyRs surprisingly included J urkat T-lymphocytes (Table I). This result is consistent with the negligible [3H]ryanodine binding displayed by J urkat cells (data not shown). These data contrast with other studies, which suggested that RyRs were expressed in these cells (16, 17). The explanation for our contrasting data is unclear but may reflect variable RyR expression between different J urkat cell clones.

The two RyR-positive nonexcitable cell types, HeLa and LLC-PK1 cells, expressed RyR2 and RyR3, respectively. RyR expression in HeLa cells was also recently reported by Giannini et al. (7), although they identified RyR2 and RyR3 in their control HeLa cells. The expression of only RyR2 in our HeLa cells was confirmed by sequencing and restriction digestion of PCR products and may again point to a donal variation. To extend the molecular characterization of RyR expression in these tissues, we sought to obtain evidence for RyR at the protein level. HeLa and LLC-PK1 cells were found to display significant levels of [3H]ryanodine binding, although it was about 17-fold less than in rabbit brain and approximately one-third of that found in undifferentiated PC12 cells (Fig. 3).

Despite the molecular evidence and [3H]ryanodine binding data, which clearly indicate that HeLa cells express RyRs, we were unable to directly demonstrate a RyR-dependent Ca^{2+} increase in the cells (Fig. 4). Neither caffeine nor ryanodine evoked a measurable increase in [Ca^{2+}]_{i}, when applied either on their own or in combination. A similar lack of caffeine responsiveness in HeLa cells was previously shown (39, 40). However, ryanodine caused a progressive decrease in the magnitude of the Ca^{2+} signal evoked by repetitive ATP applications and a slight increase in the latency before the [Ca^{2+}]_{i} rise (Fig. 5). The decreased magnitude of the Ca^{2+} signals is consistent with the previously described use-dependent block of RyR function by ryanodine (34, 41, 42), whereby the ryanodine-bound receptors remain in a constitutively open low conductance state. The finding that ryanodine inhibited ATP-induced Ca^{2+} signals in a use-dependent manner suggests that hormonal stimulation of HeLa cells brings about activation of RyRs to amplify the normal inositol 1,4,5-trisphosphate-dependent elevation of Ca^{2+}.

Just how this channel opening is achieved is unknown, but possibilities include activation via Ca^{2+}-induced Ca^{2+} release (8) or production of a RyR-sensitizing ligand such as cyclic adenosine diphosphate-ribose (43).

The apparent paradox between the effects of ryanodine and caffeine suggests that HeLa cells either express a caffeine-sensitive RyR isoform or express a very low level of RyR, such that an acute opening of the RyRs does little to influence Ca^{2+}, but a prolonged RyR opening can gradually deplete the stores. The latter explanation seems to be the more likely, since we detected a low density of RyRs in the [3H]ryanodine binding studies (Fig. 3), and all RyR isoforms have been shown to be caffeine-sensitive (44). Furthermore, similar results i.e. apparent caffeine-insensitivity and a ryanodine-induced Ca^{2+} store depletion, have been reported by Giannini et al. (3) for mink lung epithelial cells expressing RyR3. It seems likely that a low density of RyR may explain the lack of effect of caffeine on Ca^{2+}, in several cell types shown by molecular techniques to express RyRs (7).

The function of the RyRs expressed in nonexcitable cells is not fully established. In a few cell types, such as sea urchin eggs (29) and pancreatic acinar cells (30), RyRs may contribute to the initiation of Ca^{2+} signals. In hepatocytes, the recruitment of RyRs has been reported to be agonist-specific (9). Data from the present study suggest that RyRs in nonexcitable HeLa cells may provide a subtle regulation of the magnitude and kinetics (Fig. 5) of hormone-evoked [Ca^{2+}]_{i} responses. These data indicate that RyRs make an important contribution to intracellular Ca^{2+} signals in a variety of nonexcitable cell types.

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