Synchronized Ca$^{2+}$ signaling by intercellular propagation of Ca$^{2+}$ action potentials in NRK fibroblasts

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De Roos, Albert D. G., Peter H. G. M. Willems, Everardus J. J. van Zoelen, and Alexander P. R. Theuvenet. Synchronized Ca$^{2+}$ signaling by intercellular propagation of Ca$^{2+}$ action potentials in NRK fibroblasts. Am. J. Physiol. 273 (Cell Physiol. 42): C1900–C1907, 1997.—The intercellular propagation of Ca$^{2+}$ waves by diffusion of inositol trisphosphate has been shown to be a general mechanism by which nonexcitable cells communicate. Here, we show that monolayers of normal rat kidney (NRK) fibroblasts behave like a typical excitable tissue. In confluent monolayers of these cells, Ca$^{2+}$ action potentials can be generated by local depolarization of the monolayer on treatment with either bradykinin or an elevation of the extracellular K$^+$ concentration. These electrotonically propagating action potentials travel intercellularly over long distances in an all-or-none fashion at a speed of ~6.1 mm/s and can be blocked by L-type Ca$^{2+}$ channel blockers. The action potentials are generated by depolarizations beyond the threshold value for L-type Ca$^{2+}$ channels of about ~15 mV. The result of these locally induced, propagating Ca$^{2+}$ action potentials is an almost synchronous, transient increase in the intracellular Ca$^{2+}$ concentration in large numbers of cells. These data show that electrically coupled fibroblasts can form an excitable syncytium, and they elucidate a novel mechanism of intercellular Ca$^{2+}$ signaling in these cells that may coordinate synchronized multicellular responses to local stimuli.

bradykinin; intracellular calcium; calcium channels; normal rat kidney fibroblasts

INTERCELLULAR COMMUNICATION plays an important role in the coordination of cooperative cellular responses. In excitable cells, such as nerve and muscle cells, action potentials caused by the regenerative opening of voltage-dependent channels provide a fast (0.1–100 m/s) mechanism for signaling over long distances. In nonexcitable cells, coupling via gap junctions provides a way for intercellular signaling, and it has been shown that the intercellular diffusion of n-myo-inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] can induce Ca$^{2+}$ waves that slowly propagate (20–50 μm/s) from cell to cell (26).

Fibroblasts are considered to be a classic example of nonexcitable cells. Fibroblasts can be electrically coupled to each other (7, 15, 18, 25), and in several tissues, such as kidney, intestine, and dermis, these cells form three-dimensional communicating networks (19). Fibroblasts can also be electrically coupled to other cells, including myocytes (24) and endothelial (17) and neuronal (6) cells. Electrical coupling via gap junctions provides a way for the fast transduction of membrane potential changes between cells and can thus coordinate signaling. Although it has been reported that fibroblasts possess voltage-dependent Ca$^{2+}$ channels (3, 5, 13, 21, 22) that would in principle enable these cells to generate action potentials, a function for such channels in these cells has so far been unclear.

Monolayers of normal rat kidney (NRK) fibroblasts are electrically well coupled (7) and therefore provide a model system to investigate the intercellular transduction of electrical signals. NRK fibroblasts, clone 49F, can be made quiescent by serum deprivation of confluent cultures. When these quiescent cells are subsequently treated with epidermal growth factor (EGF) as the only growth-stimulating polypeptide, the cells undergo one additional cell cycle before they undergo density-dependent growth inhibition or density arrest (29). We recently showed that spontaneous repetitive membrane potential depolarizations were observed in ~50% of monolayer cultures of density-arrested NRK cells (9). Although these depolarizations showed the characteristics of action potentials, the spontaneous occurrence of the depolarizations in these cells did not allow a further study on the mechanism of propagation and induction of action potentials. In contrast to the density-arrested cells, quiescent NRK fibroblasts never exhibited spontaneous action potential-like depolarizations.

In the current study, we show that in quiescent NRK cells action potentials can be induced by bradykinin (BK), which allowed the study of the mechanism of propagation and induction of action potentials in these cells. BK is an agonist that not only increases intracellular Ca$^{2+}$ (1) but also depolarizes NRK fibroblasts via an increased Ca$^{2+}$-activated Cl$^-$ conductance (8). In monolayers of quiescent NRK fibroblasts, local depolarization of only a small part of the monolayer with BK can induce an intercellularly propagating Ca$^{2+}$ action potential that results in a fast transient increase in the intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) in large numbers of cells. The Ca$^{2+}$ action potentials reported here provide a mechanism for intercellular communication that is several magnitudes faster than the reported intercellular Ins(1,4,5)P$_3$-mediated Ca$^{2+}$ waves in nonexcitable cells.

EXPERIMENTAL PROCEDURES

NRK cells (clone 49F) were seeded at a density of 1.0 × 10$^4$ cells/cm$^2$ and grown to confluency in bicarbonate-buffered Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% newborn calf serum as previously described (1). The tissue culture dishes that were used had diameters of 35, 55, 85, and 140 mm, containing 2, 4, 10 and 20 ml of media, respectively. Confluent cultures were incubated in serum-free DF medium (1:1 DMEM-Ham’s F-12) supplemented with 30 nM Na$^+_2$SeO$_3$ and 10 μg/ml human transferrin for 2–4 days, by

C1900 0363-6143/97 $5.00 Copyright © 1997 the American Physiological Society
which time the cells became quiescent. DF medium is a cell culture medium that contains as main inorganic salts (in mM) 109.5 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.81 MgCl₂, 44.0 NaHCO₃, and 1.0 NaH₂PO₄ and is supplemented with essential nutrients such as glucose, amino acids, and vitamins for optimal cell growth. For patch-clamp experiments, cells were incubated in either normal Ca²⁺-containing DF medium or nominally Ca²⁺-free DF medium supplemented with the indicated concentrations of SrCl₂ and equilibrated with 5% CO₂ to a pH of 7.4. When SrCl₂ was added to the medium, the concentration of NaCl was lowered to maintain osmolarity. When ion substitutions were made, N-methyl-D-glucamine was substituted for Na⁺, whereas gluconate was substituted for Cl⁻. 1,2-Bis(2-aminophenoxy)ethane-N,N',N',N'-tetraacetate acid (BAPTA) loading was achieved by incubating the cells for 30 min in the presence of 100 μM BAPTA-acetoxymethyl ester (AM) (Molecular Probes, Eugene, OR), after which the cells were incubated in DF medium for 10 min.

Whole cell patch-clamp measurements were carried out as previously described (7). Patch pipettes were made from thin-walled glass (SG150T, Clarke Electromedical Instruments, Pangbourne, UK) using a two-stage pipette puller (L/M-3P-A, List Electronic, Darmstadt, Germany). Pipettes were filled with a solution containing (in mM) 25 NaCl, 120 KCl, 1 CaCl₂, 1 MgCl₂, 3.5 ethylene glycol-bis(β-aminoethyl ether)-N,N',N',N'-tetraacetate acid (EGTA), and 10 tris(hydroxymethyl)aminomethane (pH 7.4) and had resistances of 4–6 MΩ. Membrane potential was detected with an EPC-7 patch-clamp amplifier (List Electronic) in the current-clamp mode. Because monolayers of NRK cells are electrically well coupled, there is electrical access from the patched cell to neighboring cells (7). Therefore, the measured membrane potential will be an average of many coupled cells, and only the intracellular components of the patched cell are washed out. In this way, stable resting membrane potentials could be measured for >1 h.

Ratio fluorometry for measurements of [Ca²⁺] i was performed as previously described (31). In short, cells were loaded with 1.5 μM fura 2-AM (Molecular Probes) for 30 min and washed for 20 min in DF medium. Excitation wavelengths were 340 and 380 nm, and emission was monitored at 500 nm. Dynamic video imaging was carried out using the MagiCal hardware and Tardis software of Joyce Loebl (Tyne and Wear, UK). [Ca²⁺] i were calculated after calibration of fluorescence ratios to free [Ca²⁺] i from five individual experiments using 10 μM ionomycin and 20 mM EGTA to obtain maximal and minimal [Ca²⁺] i, respectively.

Figure 1A shows the experimental setup for most of the patch-clamp experiments. A cell from a monolayer culture was patched, and a depolarizing stimulus was given by the manual application of a small volume (5 or 10 μl) of 10 nM BK or 124 mM KCl at a certain distance from the measuring patch-clamp pipette (distant stimulus) using a 20-μl Gilson micropipette. By this mode of application, it was ensured that only a small part of the monolayer was exposed to stimulatory concentrations of the stimulus and that the membrane potential could be recorded in cells that were not directly exposed to the stimulus. Also, propagation of membrane potential signals was measured by using two patch-clamp pipettes that were located at a certain distance (700–900 μm) from each other (Fig. 1B). In this case, propagation of a signal could be determined from the delay in response between the two pipettes when a distant stimulus, similar to the one in Fig. 1A, was given on either side of each pipette.

Results

We recently showed that spontaneous repetitive membrane potential depolarizations were observed in monolayer cultures of density-arrested NRK cells (9). In contrast to those density-arrested cells, spontaneous action potential-like depolarizations were never seen in quiescent NRK fibroblasts. However, perfusion of BK to monolayers of cells could induce a fast action potential-like depolarization (spike; Fig. 2A) that preceded the sustained depolarization evoked by BK. We have previously shown that this sustained depolarization, which...
could last up to 15 min, is caused by an increase in a 
$\text{Ca}^{2+}$-activated $\text{Cl}^-$ conductance (8) due to the release of 
$\text{Ca}^{2+}$ from intracellular stores (1).

The spike preceding the long-lasting depolarization by 
$\text{BK}$ resembled an action potential, and, to investi-
gate whether action potentials could be induced in 
quiescent NRK cells by $\text{BK}$, the effect of $\text{BK}$ was 
measured distant from the site of its application in 
confluent quiescent monolayers of these cells. By the 
application of a small volume ($5 \mu l$) of $10 \text{nM BK}$ in a 
large volume of medium ($2.5-20 \text{ml}$) it was ensured 
that only a small part of the monolayer was exposed to 
stimulatory concentrations of the agonist. In cells of the 
monolayer that were located at a distance far enough to 
prevent direct activation by a stimulatory concentra-
tion of $\text{BK}$ (cf. Fig. 1A; distant stimulation), an action 
potential could be induced (Fig. 2B). This action poten-
tial could also be induced by depolarization of a small 
part of the monolayer by the distant addition of a small 
volume of an elevated extracellular $\text{K}^+$ concentration 
($[\text{K}^+]_o$ 124 mM; Fig. 2C).

A possible effect of diffusion of $\text{BK}$ to the site of 
measurement was ruled out by three facts. 1) When the 
small volume of $10 \text{nM BK}$ used for distant stimulation 
was first diluted to the final concentration after total 
diffusion in the bath, no depolarization was observed.
Therefore, the dilution that occurs with distant stimu-
lation causes substimulatory levels of $\text{BK}$ (see also Fig.
4C). Concentrations of $\text{BK} > 1 \text{nM}$ have no effect on the 
membrane potential of NRK cells (de Roos, van Zoelen, 
and Theuvenet, unpublished observations). 2) In cases 
in which no action potential was evoked by distant 
addition of $\text{BK}$ no distant depolarization was observed 
either. Also, when the propagating action potential was 
block$\text{ed}$ with $\text{felodipine}$, no direct depolarization by 
$\text{BK}$ was observed (cf. Fig. 3D). 3) A depolarization directly 
evoked by the distant application of a high concentra-
tion of $\text{BK} (5 \mu l$ of $0.5 \text{mM BK}$ at $20 \text{mm})$ resulting from a 
diffusion from the site of application to the site of 
measurement took $> 1 \text{min}$ to appear, whereas an 
action potential in this case had already been observed 
within $4 \text{s}$. This shows that diffusion of $\text{BK}$ to the site of 
measurement in a bath with no perfusion is much slower than the propagation velocity of the action 
potential (see also Fig. 4A). Therefore, it is concluded 
that the distant addition of a small volume of $\text{BK}$ or $\text{K}^+$ 
has no direct effect on the patched cell in which the 
action potential is measured.

These distantly evoked action potentials were seen in 
quiescent NRK cells in $\sim 10\%$ of the monolayer cultures 
when tested with normal $\text{Ca}^{2+}$-containing media. The 
probability of the occurrence of the action potentials 
could be increased (to $\sim 25\%$) by incubating the cells 
with the L-type $\text{Ca}^{2+}$ channel activator (10) BAY K 8644 
(Fig. 2C), indicating that L-type channels are involved 
in the generation of the action potential. However, the 
incidence of the action potentials could be markedly 
increased to almost $100\%$ when media were used in 
which $\text{Ca}^{2+}$ was replaced by $\text{Sr}^{2+}$. In this respect, 
quiescent NRK cells differ from density-arrested cells 
in which spontaneous action potentials occur in physi-
ological media.

The ability of $\text{Sr}^{2+}$ to substitute for $\text{Ca}^{2+}$ indicated a 
role for extracellular $\text{Ca}^{2+}$ influx through $\text{Ca}^{2+}$ chan-
nels, since it is well known that $\text{Sr}^{2+}$ can permeate $\text{Ca}^{2+}$ 
channels (16). Because action potentials could be consis-
tently generated in media in which $\text{Sr}^{2+}$ substituted for 
$\text{Ca}^{2+}$, we decided to use $\text{Sr}^{2+}$-containing media to study 
the mechanism underlying the generation and propaga-
tion of the action potential. An additional benefit of 
$\text{Sr}^{2+}$-containing media is the better solubility of $\text{Sr}^{2+}$ in 
bicarbonate-based buffers, thus allowing an increase in 
the divalent cation concentration in the medium. $\text{Ba}^{2+}$ 
could not be used as a charge carrier, since $\text{Ba}^{2+}$ blocks 
$\text{K}^+$ channels (16) and thus depolarized NRK cells (not shown).

Figure 3A shows the effect of direct stimulation by 
$\text{BK}$ on the membrane potential of quiescent NRK 
fibroblasts in $\text{Sr}^{2+}$-containing media. Also, in these 
media, perfusion of $\text{BK}$ to monolayers induced a fast 
depolarizing spike (cf. Fig. 2A) followed by a sustained 
depolarization. The distant stimulation by $\text{BK}$ induced 
an action potential (Fig. 3B) similar to the ones in 
$\text{Ca}^{2+}$-containing media, although the peak values of the 
action potential became more pronounced when the 
cells were incubated in media containing elevated 
concentrations of $\text{Sr}^{2+}$. This action potential was charac-
terized by a fast upstroke followed by a plateau phase, 
after which membrane potential quickly returned to 
resting levels. The duration of the action potential 
(measured from onset to repolarization to $\sim 50 \text{mV}$) 
varied from monolayer to monolayer, with values be-
tween 4 and 35 s and a mean (± SE) of 16.2 ± 8.3 s (n = 34). Once initiated, the action potential propagated in an all-or-none fashion, independently of the distantly applied stimulatory concentration of either K+ or BK, characteristic of an action potential (see also Fig. 4B).

The L-type Ca2+ channel blocker felodipine (27, 30) completely blocked the fast transient spike in cells located at the site of application of BK without affecting the sustained depolarization (Fig. 3C). The action potential was also blocked by the L-type Ca2+ channel blocker nifedipine (27, 28) (not shown). These results indicate that opening of L-type Ca2+ channels is responsible for the spike depolarization but not for the sustained depolarization caused by BK. Moreover, felodipine and nifedipine completely blocked the action potential in cells that were not directly exposed to BK (Fig. 3D), showing that opening of L-type Ca2+ channels is necessary for the appearance of the action potential.

To determine the conduction velocity of the propagating action potential, action potentials were induced at several distances from the recording pipette (cf. Fig. 1A), and the resulting delay was measured. Figure 4A shows that this delay increased linearly with the distance and corresponds to a conduction velocity of 6.1 mm/s (r² = 0.994). At all distances tested, the shape of the action potentials was similar, showing that the action potentials was similar, showing that the propagation of the action potential was also blocked by the L-type Ca2+ channel blocker nifedipine (27, 28) (not shown). These results indicate that opening of L-type Ca2+ channels is responsible for the spike depolarization but not for the sustained depolarization caused by BK. Moreover, felodipine and nifedipine completely blocked the action potential in cells that were not directly exposed to BK (Fig. 3D), showing that opening of L-type Ca2+ channels is necessary for the appearance of the action potential.

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The apparent involvement of L-type Ca2+ channels that emerges from the findings in Figs. 2 and 3 pointed to a direct role of divalent cation influx through Ca2+ channels in the generation of the action potential. Consequently, it may be expected that the peak value of the action potentials will be sensitive to changes in the external divalent cation concentration. The dependence of the peak potential on [Sr2+]e is shown in Fig. 5A. The peak potential increased linearly with the logarithm of [Sr2+]e. The slope of the graph is 29 mV/10-fold increase (r² = 0.996) in the [Sr2+]e, as expected for a Sr2+ electrode (16). This indicates that the spike depolarization is solely caused by increased Sr2+, and therefore Ca2+, permeability.

L-type Ca2+ channels are activated by depolarizations beyond a threshold value of −10 to −20 mV (16, 28). Because well-coupled monolayers cannot be uniformly voltage clamped (2), increasing concentrations of K+ were perfused to the cells to progressively depolarize the cells. Figure 5B shows that only concentrations of K+ that depolarized the cells beyond −15 mV were able to produce the all-or-none action potentials, which is similar to the reported threshold value of L-type Ca2+ channels.
Channel blockers (16) tetrodotoxin (Fig. 6C; duration of action potential, 19.9 ± 12.3 s; peak, 34.9 ± 13.7 mV; n = 5), and neither did the potent Na+ channel blocker (16) tetrodotoxin (Fig. 6C; duration of action potential, 14.2 ± 10.7 s; peak, 30.8 ± 4.8 mV; n = 4), which demonstrates that there is no Na+ component to the action potential. Low-Cl− media (Fig. 6D) changed the shape of the plateau phase of the action potential without affecting the duration (11.6 ± 2.2 s; n = 4) and slightly affected the peak of the depolarizing spike peak (15.8 ± 3.5 mV; n = 4), showing that the plateau phase is caused by an increase in Cl− conductance of the plasma membrane. Buffering [Ca2+]i by loading the cells with the Ca2+ chelator BAPTA abolished the plateau phase (Fig. 6E; duration 1.6 ± 0.4 s; peak 25.4 ± 7.5 mV; n = 9). These results indicate that the plateau phase involves a Ca2+-activated Cl− conductance. The overall effect of a Ca2+-activated Cl− conductance is therefore a lengthening of the Ca2+ action potential. In BAPTA-loaded cells, action potentials could still be induced by a high [K+]o but not by BK, showing that the depolarization by BK is [Ca2+]i dependent. In summary, these results show that the upstroke of the action potential is solely mediated by a Ca2+ influx and is therefore a Ca2+ action potential (12), whereas the plateau phase is caused by an increased Ca2+-mediated Cl− conductance.

The involvement of L-type Ca2+ channels was further supported by the finding that action potentials could be induced by the distant stimulation with the L-type Ca2+ channel activator BAY K 8644 (10) (Fig. 6F). Thus, in Ca2+-containing media, incubation with BAY K 8644 favors the induction of action potentials by a depolarizing stimulus (Fig. 3C), but in Sr2+-containing media BAY K 8644 can evoke action potentials by itself, indicating an increased activity of L-type Ca2+ channels in Sr2+-containing media.

The involvement of voltage-dependent Ca2+ channels in the action potential implied an influx of Ca2+ (or Sr2+ when this ion was used as a charge carrier) through these channels during an action potential and therefore an increase in [Ca2+]i or [Sr2+]i. Dynamic video imaging with the fluorescent probe fura 2 was used to simultaneously monitor the influx of Sr2+ or Ca2+ of ~100 cells of the monolayer. Fura 2 can be used to measure not only Ca2+ influx but also Sr2+ influx, although the affinity of fura 2 for Sr2+ is lower than that for Ca2+ (20). Figure 7A shows the average increase in [Ca2+]i in all measured (~100) cells after the distant induction of an action potential in normal, Ca2+-containing me-
In animal cells, action potentials depend on a regenerative depolarization caused by voltage-dependent ion channels that open on depolarization. Depolarization with either BK or high extracellular K⁺ evoked action potentials in NRK cells. Because the resting membrane potential is determined by a K⁺ conductance and is therefore dependent on the concentration gradient of K⁺, elevating the extracellular K⁺ concentration directly depolarizes the cell. BK, however, releases Ca²⁺ from intracellular stores, leading to an increase in [Ca²⁺]ᵢ, which opens a Ca²⁺-activated Cl⁻ conductance with a concomitant depolarization (8). Therefore, by opening a Cl⁻ conductance via an increase in [Ca²⁺]ᵢ, BK indirectly depolarizes the cells. BK induces a propagating action potential but, in contrast to high K⁺, does not induce an action potential in cells directly exposed to BK (de Roos et al., unpublished observations).

**DISCUSSION**

The finding that monolayers of fibroblasts are excitable elucidates a new aspect of fundamental importance in the signaling of these cells, since these cells were hitherto considered to be a classic example of nonexcitable cells (4, 14, 23). The present data show that local application of physiological concentrations of an agonist like BK can induce a fast transient increase in [Ca²⁺]ᵢ in large numbers of cells that were not directly exposed to the stimulus. The Ca²⁺ action potentials reported here provide a mechanism for intercellular communication that is several orders of magnitude faster than the reported Ins(1,4,5)P₃-dependent intercellular Ca²⁺ waves in fibroblasts and nonexcitable cells (20–50 μm/s; see Ref. 26), although slower than action potentials in nerve (1–100 m/s) or muscle (0.1–1 m/s), probably due to the electrical parameters of the fibroblast monolayer and the channels involved (16).

Electrical coupling between cells is a prerequisite for the intercellular propagation of action potentials. Fibroblast (or fibroblast-like) cells can be electrically coupled to each other (15, 18, 25) and may even form threedimensional communicating networks in vivo (19). Fibroblasts can also be electrically coupled to other cells (17), and in this way networks of fibroblasts may play a central role in the synchronous or coordinated behavior of tissues. For example, fibroblasts are electrically coupled to myocytes and can transmit action potentials (24), but fibroblasts can also rescue excitability of dysgenic myotubes (6). We hypothesize that fibroblasts play an active role in the initiation and transduction of electrical signals involved in the coordination of multicellular activities.

In animal cells, action potentials depend on a regenerative depolarization caused by voltage-dependent ion channels that open on depolarization. Depolarization with either BK or high extracellular K⁺ evoked action potentials in NRK cells. Because the resting membrane potential is determined by a K⁺ conductance and is therefore dependent on the concentration gradient of K⁺, elevating the extracellular K⁺ concentration directly depolarizes the cell. BK, however, releases Ca²⁺ from intracellular stores, leading to an increase in [Ca²⁺]ᵢ, which opens a Ca²⁺-activated Cl⁻ conductance with a concomitant depolarization (8). Therefore, by opening a Cl⁻ conductance via an increase in [Ca²⁺]ᵢ, BK indirectly depolarizes the cells. BK induces a propagating action potential but, in contrast to high K⁺, does not induce an action potential in cells directly exposed to BK (de Roos et al., unpublished observations).
tions); transduction of the depolarization to neighboring cells seems to be the trigger for the propagating action potential. The action potential induced by perfusing BK to the bath (Figs. 2A and 3A) is a combination of distant stimulation (when BK enters the bath), which evokes an action potential, and direct stimulation (when BK reaches the cells), which causes the long-lasting depolarization.

The following mechanism for the generation of action potentials is proposed. Depolarization by extracellular K+ directly depolarizes the cells beyond the threshold value for L-type Ca2+ channels, causing these channels to open. In the case of BK, transduction to neighboring cells of a depolarization caused by the opening of Ca2+-activated Cl- channels activates L-type Ca2+ channels in these cells. Opening of Ca2+ channels generates an influx of Ca2+ in the cells, with a concomitant subsequent depolarization toward the equilibrium potential for Ca2+. Further transduction of this depolarization to neighboring cells via gap junctions results in the regenerative opening of Ca2+ channels in these cells, resulting in active propagation of the signal through the whole monolayer. The influx of Ca2+ during the action potential opens a Ca2+-activated Cl- conductance responsible for the plateau phase. Because the [Ca2+]i seems to play a role in the maintenance of the plateau phase of the action potential, simultaneous measurements of [Ca2+]i and membrane potential are needed to determine a possible threshold function of the [Ca2+]i.

The following evidence for the involvement of L-type Ca2+ channels was obtained: 1) the action potentials could be blocked by the well-characterized L-type Ca2+ channel blockers felodipine and nifedipine (27, 28, 30); 2) action potentials could be induced by the L-type Ca2+ channel activator BAY K 8644 (10, 28); and 3) the observed threshold value for opening corresponds well with the reported value for L-type Ca2+ channels in the literature (16, 28). Moreover, we recently showed directly by means of voltage-clamp measurements in single trypsinized NRK cells that these fibroblasts indeed possess L-type Ca2+ channels (9).

L-type Ca2+ channels have also been shown to be present in mouse BALB/c 3T3 (21), human foreskin (3), mouse Swiss 3T3 (5), and in REF52 fibroblasts (14), whereas in Swiss 3T3 cells T-type Ca2+ channels have also been found (22). So far, a function for these voltage-dependent Ca2+ channels in fibroblasts has been unclear. Our results suggest that they may function in the generation and propagation of action potentials.

L-type Ca2+ channels are inhibited by high [Ca2+]i (11), and Ca2+ action potentials are more easily evoked under conditions of low [Ca2+]i (12, 16). When Sr2+ was used as the charge carrier, with no Ca2+ added to the medium, action potentials in quiescent NRK cells could be consistently generated by exposure to either BK or an elevated [K+]. Therefore, these Sr2+-containing media have been used in this study to investigate the induction and propagation of action potentials in NRK cells. It is hypothesized that incubation of the cells in low-Ca2+ media, which results in a decrease of the [Ca2+]i, could be responsible for the loss of inhibition of the L-type Ca2+ channels by [Ca2+]i, causing them to open more easily. A block of the action potentials by high [Ca2+]i would be in agreement with the observation that the addition of a small volume of K+ directly to cells evokes an action potential in these cells, whereas depolarization by the local addition of a small volume of BK, which first causes an increase in the [Ca2+]i, does not evoke an action potential in the cells directly exposed to BK (de Roos et al., unpublished observations) but does evoke a propagating action potential in the remainder of cells that were not directly exposed to BK (this article).

The present findings show that activation of L-type Ca2+ channels can evoke action potentials in NRK fibroblasts. The action potentials reported here are similar to the spontaneous action potential-like depolarizations (9) seen in NRK cells that are grown to density arrest in the presence of EGF (1, 29), and it is concluded that Ca2+ action potentials underlie these spontaneous depolarizations. The cells used in this study to induce action potentials were made quiescent by the deprivation of serum, whereas density-arrested cells stop growing when they reach a critical cell density, similar to the growth inhibition of cells in vivo (29). Thus the ability of NRK fibroblasts to behave like an excitable tissue may depend on the appropriate environmental cues. The fact that density-arrested NRK monolayers exhibit spontaneous action potentials in normal Ca2+-containing media may imply an increased activity of L-type Ca2+ channels at higher cell densities, and thus that activity of these Ca2+ channels is modulated by cell density. A role of the activity of Ca2+ channels in the incidence of action potentials is in agreement with the observation that, in Ca2+-containing media, occurrence of action potentials in quiescent cells was markedly increased when they were incubated with the L-type channel activator BAY K 8644.

We thank Drs. H. Jongsma, M. Rook, and D. Ypey for helpful discussions. Dr. M. J. Berridge for critical reading of an earlier version of the manuscript, and P. Peters for technical support.

This research was supported by the Netherlands Foundation for Life Sciences (NWO-SLW).

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Received 11 April 1997; accepted in final form 2 September 1997.

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