—Originals—

Evidence for norepinephrine-activated Ca^{2+} permeable channels in guinea-pig hepatocytes using a patch clamp technique

Tomoo Nagano[1], Ryoichi Sato[2], Hiroyuki Matsuda[1] and Takumi Aramaki[1]

[1]First Department of Internal Medicine, Nippon Medical School, and [2]Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School

Abstract

To determine whether the hepatocyte plasma membrane possesses a Ca^{2+} channel, we applied a patch clamp technique to isolated guinea-pig hepatocytes. In a cell-attached configuration, using an internal pipette solution of 110 mM $BaCl_2$ or $CaCl_2$, we observed sporadic inward single channel currents (Po=0.004±0.002, n=6) at various membrane potentials. The unit amplitude was 0.60±0.15 pA (n=6) at resting membrane potential. The single channel conductance was 20.4±4.6 pS (n=6) and this channel showed no rectification and no voltage dependence. Bay K 8644, a dihydropyridine Ca^{2+} channel activator, did not affect this channel activity. Although norepinephrine in the pipette solution did not activate this channel, its external application increased channel activity. These observations suggest that guinea-pig hepatocytes possess Ca^{2+} permeable channels that differ from the voltage-operated Ca^{2+} channels found in excitable cells and that such channels are responsible for the agonist-stimulated Ca^{2+} entry in hepatocytes. (J Nippon Med Sch 1999；66：127—133)

Key words：patch clamp technique, guinea-pig hepatocyte, Ca^{2+} channel, norepinephrine

Introduction

Calcium mobilizing hormones such as norepinephrine, vasopressin, and angiotensin II accomplish signal transduction in hepatocytes by increasing the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$)[1]. An increase in $[Ca^{2+}]_i$ results from an inositol 1, 4, 5-trisphosphate ($Ins\text{-}P_3$)-mediated Ca^{2+} release from internal stores, such as endoplasmic reticulum (first phase) and from a transmembrane Ca^{2+} influx (second phase), which is important to the maintenance of an elevated $[Ca^{2+}]_i$ level[2].

The Ca^{2+} influx across the plasma membrane in response to stimulation by an agonist is termed a receptor-activated Ca^{2+} inflow system (RACIS)[3]. Receptor-operated Ca^{2+} channels have been demonstrated in other non-excitable cells by means of electrophysiological techniques. Recent studies have shown that the vascular endothelial cells[4], T-lymphocytes[5] and mast cells[6] all possess receptor-operated Ca^{2+} channels and that these channels are responsible for the agonist-stimulated Ca^{2+} influx. While the mechanism underlying the RACIS in hepatocytes has not yet been fully resolved, increasing evidence suggests the existence of a Ca^{2+} channel in hepatocytes[7,8]. To investigate whether the hepatocyte plasma membrane possesses a Ca^{2+} channel, we applied the patch clamp technique[9] to isolated guinea-pig hepatocytes and succeeded in recording an inward Ca^{2+} current as a single channel current and in observing the augmentation of this current by norepinephrine. Ca^{2+} permeable channels demonstrated in this study might be an explanation for the RACIS in

Corresponding to Tomoo Nagano, First Department of Internal Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan

hepatocytes. We report here the nature of Ca^{2+} permeable channels which exist in a guinea-pig hepatocyte plasma membrane.

Materials and Methods

(1) *Cell preparation*

Isolated hepatocytes were prepared from guinea-pig livers using the collagenase perfusion method developed by Berry et al.[10] Briefly, female Hartley guinea-pigs (300～400 g) were anesthetized by intraperitoneal injection of pentobarbitone (60 mg/kg). The abdominal wall was incised and the portal vein was cannulated. The liver was first perfused with a Ca^{2+}-free perfusion solution that contained ethyleneglycol-bis-N, N, N', N', -tetraacetic acid (EGTA) and was then switched to the second solution that contained Ca^{2+} and collagenase (0.5 mg/m*l*). After this treatment, the liver was excised and minced in Hanks' balanced salt solution (BSS). The minced tissue was filtered through a 150 μm nylon mesh to remove fibrous tissues, and washed 3 times by centrifugation at 50 g for 3 min. The cell pellets were resuspended in Hanks' BSS. The cells were used within 6h after isolation. The viability of the isolated cells was assessed by the Trypan Blue exclusion technique after the isolation procedure. The preparation was discarded if < 80% of the cells excluded Trypan Blue.

(2) *Recording of single channel currents*

Single channel patch clamp experiments were performed in the cell-attached conditions according to the method of Hamil et al.[9] using an AXOPATCH-1 D amplifier (Axon Instrument, Inc., Foster City, CA, USA). The hepatocytes were seeded on a microscope coverslip placed in a perfusion chamber that contained an external solution. The chamber was mounted on the stage of an inverted phase-contrast microscope (DIAPHOTO-TMD, Nickon, Tokyo). Glass microelectrodes were fabricated from glass capillary tubes using a two-stage pipette puller (type PP-83, Narishige Scientific Instruments Laboratories). The electrodes had a resistance of 6～8 MΩ after their tips had been heat polished and they were filled with an internal solution. A microelectrode was positioned against the cells using a three-dimensional micromanipulator, and a 10 to 50 GΩ seal was established by the application of gentle suction to the electrode. To maintain the pipette potential at ground level, an Ag-AgCl wire was connected to the bathing solution via an agar bridge. Current signals were monitored continuously with a conventional thermowriting pen recorder (Recti-Horiz-8 K ; San-ei, Japan) and stored on a personal computer (COMPAQ DESKPRO 386 S) for subsequent analysis with pCLAMP (version V) software (Axon Instruments). Channel openings were defined using the 50 percent threshold criterion. The open state probability was determined by a manual analysis of current traces as a fraction of the total time that the channels were open. The open time histogram was fitted to an exponential curve using a least-squares program. All data were filtered through an eight-pole Bessel filter at 1 KHz. Averaged values are expressed as mean ± SD. All the experiments were performed at room temperature.

(3) *Solutions and drugs*

The first solution perfused via the hepatic portal vein cannulation contained (in mM) : 140 NaCl, 5.4 KCl, 0.6 NaH_2PO_4, 0.4 Na_2HPO_4, 0.5 EGTA, 4.2 $NaHCO_3$, 5 Glucose, 9.6 N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid (HEPES) ; adjusted to pH 7.2 with NaOH. The second perfusion solution contained (in mM) : 140 NaCl, 5.4 KCl, 4.0 $CaCl_2$, 0.6 NaH_2PO_4, 0.4 Na_2HPO_4, 4.2 $NaHCO_3$, 9.6 HEPES, 0.5 mg/d*l* collagenase ; adjusted to pH 7.5 with NaOH. Hanks'BSS contained (in mM) : 140 NaCl, 5.4 KCl, 1.3 $CaCl_2$, 1.0 $MgCl_2$, 1.7 $MgSO_4$, 0.6 NaH_2PO_4, 0.4 Na_2HPO_4, 4.2 $NaHCO_3$, 5 Glucose ; adjusted to pH 7.5 with $NaHCO_3$. The patch pipettes were filled with internal solutions that contained 110 mM $BaCl_2$ or 110 mM $CaCl_2$ with 8 mM HEPES. The pH of the internal solution was adjusted to 7.4 with TEA (tetraethylammonium) OH. TEAOH was used to block the potassium channels[11]. The bath solution contained (in mM) : 142 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$, 8 HEPES, 11 Glucose (pH was adjusted to 7.3 with NaOH). Norepinephrine was dissolved in the bath or the internal solution. Bay K 8644[12], a dihydropyridine Ca^{2+} channel activator, was dissolved in the internal solution. Both chemicals and collagenase were purchased from Sigma Chemical Co., St. Louis.

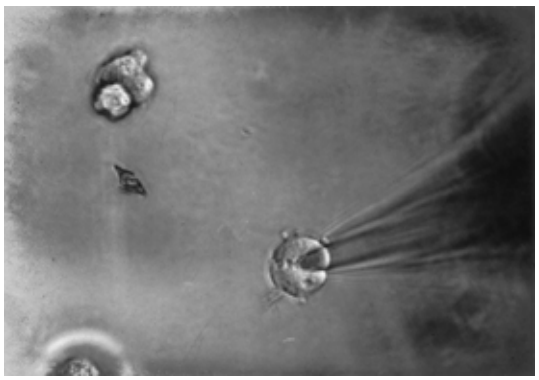

Fig. 1 A microphotograph of the isolated hepatocyte with a glass pipette attached to the cell surface. The hepatocyte is round and has a diameter of about 20 μm.

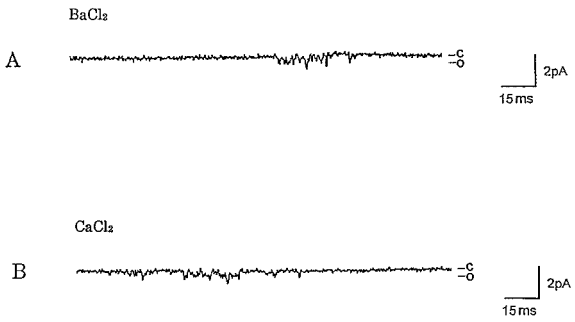

Fig. 2 A Inward Ba^{2+} currents observed in a cell attached patch at resting membrane potential. Downward deflections imply inward currents. Brief, sporadic openings are recorded. The closed (c) and open (o) states are indicated. B Inward Ca^{2+} currents observed under the same condition as Fig. 2. A. 110 mM $CaCl_2$ was used for the internal solution. In this experiment, current amplitudes and channel kinetics are almost the same as the Ba^{2+} currents.

Results

1. Single channel currents in a cell-attached patch with 110 mM $BaCl_2$ internal solution

Single channel activities were recorded under the cell-attached patch condition at various membrane potentials. A microphotograph of the isolated hepatocyte and an attached glass pipette is shown in **Fig. 1**. 110 mM $BaCl_2$ was used as the pipette solution. A typical example of the currents recorded at resting membrane potential is shown in **Fig. 2 A**. After a gigaohm seal had been established between the glass pipette and the hepatocyte plasma membrane, inward unitary currents (downward deflection) were observed. This channel showed brief sporadic openings. Channel activity was observed over the entire voltage range studied. The unit amplitude was approximately 0.6 pA (0.60 ± 0.15 pA, n=6) at resting membrane potential. The open probability was very low ($Po=0.004 \pm 0.002$, n=6) in the basal state, i.e. in the absence of Ca^{2+} mobilizing hormones. These inward currents were considered to be carried by the Ba^{2+} ions going from the pipette into the cytoplasm, because Ba^{2+} was the only charge carrier with an inwardly directed driving force at negative resting membrane potential. Similar current amplitudes and channel kinetics were observed when the Ca^{2+} ions were substituted for the Ba^{2+} ions in the pipette solution (**Fig. 2 B**). This finding suggests that the Ba^{2+} ions pass through a common pathway that allows the flow of Ca^{2+} into the cytoplasm, i.e. through a Ca^{2+} permeable channel.

2. Characteristics of Ca^{2+} permeable channel currents

The current-voltage (I-V) relationship for this channel, recorded with 110 mM $BaCl_2$ pipette solution is shown in **Fig. 3**. The I-V relationship was linear and did not show any rectification at the observed potential range. The single channel conductance was about 20 pS, (20.4 ± 4.6 pS, n=6) as calculated from the slope of the current-voltage relationship. By extrapolation of this slope, the estimated reversal potential was approximately +30 mV. While the single channel amplitude was dependent on membrane potential, channel gating behavior did not appear to be affected by either depolarizing or hyperpolarizing voltage jumps. There was no qualitative change in channel appearance at different membrane potentials. Kinetic parameters were analyzed to evaluate the voltage-sensitivity of the channel gating. The open probability was so low in these experiments that we analyzed only the open time. The open time histogram for this Ca^{2+} permeable channel during burst at resting membrane potential was fitted by a single exponential function and revealed a mean open time of 1.3 ± 0.4 ms (n=6). This result indicates the existence of one open state for this Ca^{2+} permeable channel. Next, we performed a similar kinetic analysis at differing membrane potentials. As shown in **Fig. 4**, kinetic parameters were not affected by a change in membrane potential. We, therefore, considered that the channel ki-

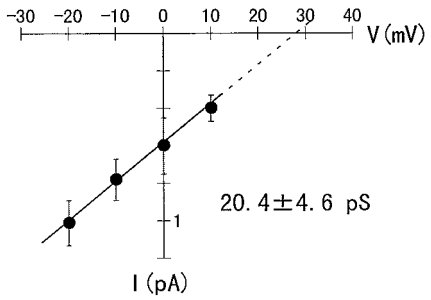

Fig. 3 Current (I) -voltage (V) relationship of the Ca^{2+} permeable channel. Membrane potential is expressed as voltage deviation from resting membrane potential. Data points represent means ± SD (n = 6). The slope conductance was 20.4 ± 4.6 pS. The extrapolated reversal potential was approximately +30 mV.

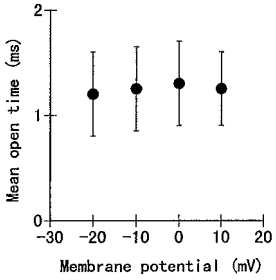

Fig. 4 Relationship between mean open time and membrane potential. Membrane potential is expressed as voltage deviation from resting membrane potential. The vertical bar through each point represents the SD (n=6 for each). The mean open time was not affected by a change in membrane potential.

netics showed no voltage dependence.

We next investigated the effect of Bay K 8644 on channel activity. When 5 μM Bay K 8644 was added to the internal pipette solution in the cell-attached patch condition, we observed no significant effect on channel gating behavior (data not shown). This observation suggests that the hepatocyte Ca^{2+} permeable channel differs from the voltage-operated Ca^{2+} channel seen in excitable cells such as cardiac myocytes and neurons.

3. Effect of norepinephrine on hepatocyte Ca^{2+} permeable channel

The above results showed that the guinea-pig hepatocyte plasma membrane contains a Ca^{2+} permeable channel. The next question was whether this channel might serve as a pathway for Ca^{2+} inflow in the hormone-stimulated state. Norepinephrine, an α_1-adrenergic agonist, is known to induce a rise in intracellular Ca^{2+} concentration in hepatocytes and to stimulate cellular physiological responses. We, therefore, investigated whether this channel could be a pathway for Ca^{2+} inflow when an increase in intracellular Ca^{2+} concentration is induced in response to norepinephrine. A representative recording is shown in **Fig. 5**. This current trace was recorded under the cell-attached condition with 110 mM $BaCl_2$ internal solution. The patch membrane was held at resting membrane potential. Before the addition of norepinephrine, this patch showed virtually no channel activity in the control bath solution. However, the addition of 20 μM norepinephrine to the bath led to a gradual activation of inward currents. This activation continued for several minutes, then subsided gradually in the presence of norepinephrine. The unit amplitude of this inward current did not differ from that observed in the control bath solution. The open probability was increased to 0.097 in this experiment.

We next investigated the effect on channel activity in the presence of norepinephrine in the internal solution. The upper trace (**Fig. 6 A**) represents the recording of inward Ba^{2+} currents at resting membrane potential in the absence of norepinephrine under the cell-attached patch condition, while the lower trace (**Fig. 6 B**) was obtained in the presence of 20 μM norepinephrine in the pipette solution. The presence of norepinephrine in the pipette solution did not affect channel activity, which suggests that norepinephrine exerted its effect via an intracellular second messenger, not by a direct interaction with α_1-adrenergic receptor.

Discussion

In hepatocytes, $[Ca^{2+}]_i$ is maintained at very low concentrations ranging from 100 to 200 nM. In contrast, the extracellular Ca^{2+} concentration is about four orders of magnitude higher than $[Ca^{2+}]_i$. Ca^{2+} is known to be one of the most important regulators of liver cell functions. Ca^{2+} mobilizing hormones such as norepinephrine, vasopressin and angiotensin II require an increase in $[Ca^{2+}]_i$ for the transduction of extracellular signals. Experiments have showed that, in the absence of Ca^{2+} in the external solution, the increase of $[Ca^{2+}]_i$ induced by hormonal stimulation is transient,

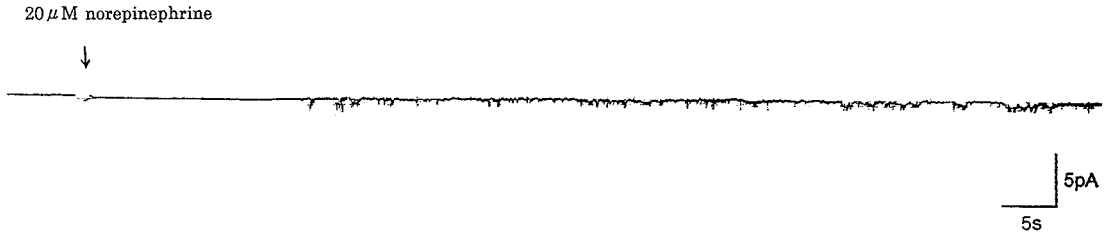

Fig. 5 Current trace showing the response to the external application of norepinephrine (20 μM). This patch showed virtually no channel activity in the control bath solution. However, channel activation was observed following the addition of norepinephrine into the bath. The arrow indicates the addition of norepinephrine. Open probability was increased to 0.097 in this experiment.

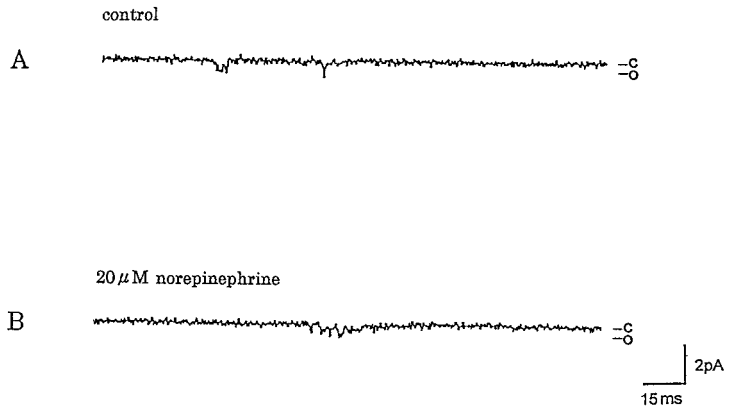

Fig. 6 Effect on channel activity of norepinephrine in the pipette solution. Upper trace (A) shows the recording of inward Ba^{2+} currents under the control condition at resting membrane potential. Lower trace (B) shows the recording in the presence of 20 μM norepinephrine in the pipette solution. Norepinephrine appeared to have no effect on channel activity.

and that the maintenance of a high [$Ca^{2+}]_i$ requires a transmembrane Ca^{2+} influx from the external solution[13]. Although it has been hypothesized that the hepatocyte RACIS is a Ca^{2+} channel, there had been no direct evidence to confirm the existence of a Ca^{2+} channel, and the mechanism of the transmembrane Ca^{2+} inflow system in hepatocytes was not understood for a long time[14]. However, several recent electrophysiological studies have suggested the existence of Ca^{2+} channels that are responsible for transmembrane Ca^{2+} influx in hepatocytes. Using a patch clamp technique, Bear et al.[7] demonstrated a Ca^{2+} permeable channel in rat hepatoma cells that is activated by extracellular ATP, and reported that this channel is identical to the non-selective cation channel that is permeable to Na^+, K^+ and Ca^{2+}. This cation channel is not affected by membrane potential and is activated by membrane stretch[15]. Duszynski et al.[8], using the whole cell patch clamp technique, reported an inward Ca^{2+} current induced by vasopressin in rat hepatocytes.

The present study is the first to demonstrate the existence of a norepinephrine-activated Ca^{2+} permeable channel in the guinea-pig hepatocyte plasma membrane using a single channel recording technique. This Ca^{2+} permeable channel exhibited a single channel conductance of about 20 pS and a nearly linear current-voltage relationship. Channel kinetics was not affected by a change in membrane potential. The previous studies that investigated the change in intracellular Ca^{2+} concentration before the use of the patch clamp technique showed conflicting results on the voltage dependence of the putative Ca^{2+} channel in hepatocytes. For example, Savage et al.[16] concluded that the Ca^{2+} channels responsible for the vasopressin-stimulated Ca^{2+} influx were closed by the depolarization of the plasma membrane by a high K^+ medium in rat hepatocytes. In contrast, Zhang et al.[17] showed that the vasopressin-induced Ca^{2+} influx was independent of the electrical potential gradient across the

plasma membrane in rat hepatocytes. Although the reason for the discrepancy between those results is unclear, our present investigation which uses a single channel recording technique suggests that the activation of the Ca^{2+} channel responsible for the hormone-stimulated Ca^{2+} influx is independent of membrane potential.

The Ca^{2+} permeable channel demonstrated in this study had a very low open probability (Po=0.004) in the basal state, and less than 50 percent of the patches that accomplished gigaohm seals contained this channel. This channel, therefore, seems to have a low channel density. In addition to its insensitivity to membrane potential, with respect to these characteristics, this channel resembled the Ca^{2+} permeable channel that was demonstrated by Kuno et al.[18] in human clonal T-lymphocyte that was activated by the mitogenic lectin, phytohaemagglutinin. It is not surprising that the hepatocytes would demonstrate a mechanism similar to that of T-lymphocytes with respect to transmembrane Ca^{2+} influx, because they are also electrically non-excitable.

The increase in channel activity obtained by exposing the cell to norepinephrine was transient and the activity was gradually decreased in the time course (**Fig. 5**), which suggests that some protective mechanism may close this channel to prevent an excessive increase in $[Ca^{2+}]_i$ and guard against cellular damage produced by calcium overload[19]. Ca^{2+} itself may be one of such mechanisms. In fact, Poggioli et al.[20] reported that intracellular Ca^{2+} inhibits Ca^{2+} influx in rat hepatocytes.

The gradual inactivation of this channel may be synchronous with Ca^{2+} oscillation (Ca^{2+} wave) previously observed in the hepatocytes stimulated by Ca^{2+} mobilizing hormones [21,22].We did not measure $[Ca^{2+}]_i$ in this experiment and could not observe the channel response to norepinephrine over prolonged periods because of the difficulty in maintaining a good recording condition due to the fragility of patch membranes or to channel rundown. Therefore, we did not evaluate the relationship between channel activation (or inactivation) and Ca^{2+} oscillation.

We observed no effect of Bay K 8644, a dihydropyridine Ca^{2+} channel activator, on channel activity. This result agrees with previous reports that Ca^{2+} influx into the hepatocytes is not inhibited by exposure to a low concentration of Ca^{2+} antagonists such as nifedipine, verapamil or diltiazem that block the voltage-operated Ca^{2+} channels[23,24]. In addition, using a patch clamp technique, several investigators[25,26] reported a failure to detect the voltage-operated Ca^{2+} channels in hepatocytes. Thus, Ca^{2+} permeable channels demonstrated in hepatocytes are considered to belong to a class that differs from the voltage-operated Ca^{2+} channels found in excitable cells such as cardiac myocytes and neurons.

The molecular mechanism involved in the opening process of the hepatocyte Ca^{2+} channel has not been established. There are at least three possible mechanisms concerning the receptor-activated Ca^{2+} entry. The first, that there exists a direct channel activation via a hormone receptor, is unlikely in this case, because the addition of norepinephrine to the pipette solution did not activate the channel, despite the activation of α_1-adrenergic receptor. A second possibility is that an intracellular second messenger that is generated by hormonal stimulation activates the channel (second messenger-activated Ca^{2+} channel). In other cell types, Kuno et al.[27] found that Ins-P_3 activates a Ca^{2+} conductance in T-lymphocyte plasma membrane using the inside-out patch clamp study. Moreover, Lückhoff et al.[28] demonstrated a Ca^{2+} permeable channel which is activated by Inositol 1, 3, 4, 5-tetrakisphosphate in endothelial cells. A third possibility is a capacitative calcium entry[29,30], in which the activation of Ca^{2+} entry is linked to the emptying of the internal Ins-P_3 sensitive Ca^{2+} store (endoplasmic reticulum). This third hypothesis postulates that an unknown messenger, termed the calcium influx factor (CIF), is released from the endoplasmic reticulum into the cytoplasm in response to a depletion of the Ca^{2+} pool, and then diffuses to the plasma membrane and activates Ca^{2+} entry. Hansen et al.[31] and Striggow et al.[32] suggested that the second and third mechanisms cited are involved in the receptor-mediated Ca^{2+} entry in rat hepatocytes in studies using fura-2, the fluorescent Ca^{2+} indicator. Our present finding that the presence of extracellular norepinephrine outside the patch increased the channel activity in a cell-attached configuration also supports the involvement of one or both of those mechanisms. Further electrophysiological investigations are required to elucidate the minute mechanism of Ca^{2+} entry at the molecular level.

References

1. Grollman EF：Calcium Signals in the Liver. In：The Liver：Biology and Pathobiology, 2nd edition（edited by Arias IM, Jakoby WB, Popper H, Schachter D, Shafritz DA）1988；pp 777−783 Raven Press, New York.
2. De Witt LM, Putney JW：α-adrenergic stimulation of potassium efflux in guinea-pig hepatocytes may involve calcium influx and calcium release. J Physiol（Lond）1984；346：395−407.
3. Barrit GJ, Hughes BP：The nature and mechanism of activation of the hepatocyte receptor-activated Ca^{2+} inflow system. Cellular Signaling 1991；3：283—292.
4. Vaca L, Kunze DL：Depletion of intracellular Ca^{2+} stores activates a Ca^{2+} selective channel in vascular endothelium. Am J Physiol 1993；267：C 920−925.
5. Zweifach A, Lewis RS：Mitogen-regulated Ca^{2+} current of T-lymphocytes is activated by depletion of intracellular Ca^{2+} stores. Proc Natl Acad Sci USA 1993；90：6295−6299.
6. Hoth M, Penner R：Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 1992；355：353−356.
7. Bear CE, Li C：Calcium-permeable channels in rat hepatoma cells are activated by extracellular nucleotides. Am J Physiol 1991；261：C 1018−1024.
8. Duszynski J, Elensky M, Cheung JY, Tillotson DL, Lanoue KF：Hormone-regulated Ca^{2+} channel in rat hepatocytes revealed by whole cell patch clamp. Cell Calcium 1995；18：19−25.
9. Hamill OP, Marty A, Nehr E, Sakmann B, Sigworth FJ：Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 1981；391：85−100.
10. Berry MN, Friend DS：High yield preparation of isolated rat liver parenchymal cells：a biochemical and fine structural study. J Cell Physiol 1969；43：506−520.
11. Hille B：The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. J Gen Physiol 1967；50：1287−1302.
12. Schramm M, Thomas G, Towart R, Franckowiak G：Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. Nature 1983；303：535−537.
13. Hansen CA, Siemens IR, Williamson JR：Calcium entry in rat hepatocytes：Stimulation by inositol 1, 4, 5-trisphosphorothioate. In：The Biology and Medicine of Signal transduction（edited by Nishizuka Y）1990；pp. 128−133 Raven Press, New York.
14. Mauger J-P, Claret M：Calcium channels in hepatocytes. J Hepatol 1988；7：278−282.
15. Bear CE：A nonselective cation channel in rat liver cells is activated by membrane stretch. Am J Physiol 1990；258：C 421−428.
16. Savage AL, Biffen M, Martin BR：Vasopressin-stimulated Ca^{2+} influx in rat hepatocytes is inhibited in high-K^+ medium. Biochem J 1989；260：821−827.
17. Zhang Y, Duszynski J, Hreniuk S, Waybill MM, LaNoue KF：Regulation of plasma membrane permeability to calcium in primary cultures of rat hepatocytes. Cell Calcium 1991；12：559−575.
18. Kuno M, Goronzy J, Weyand CM, Gardner P：Single-channel and whole-cell recordings of mitogen-regulated inward currents in human cloned helper T lymphocytes. Nature 1986；323：269−273.
19. Schanne FA, Kane AB, Young EE, Farber JL：Calcium dependence of toxic cell death：a final common pathway. Science 1979；206：700−702.
20. Poggioli J, Mauger J-P, Guesdon F, Claret M：A regulatory calcium-binding site for calcium channel in isolated rat hepatocytes. J Biol Chem 1985；260：3289−3294.
21. Woods NM, Cuthberston KS, Cobbold PH：Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. Nature 1986；319：600−602.
22. Kawanishi T, Blank LM, Harootunian AT, Smith MT, Tsien RY：Ca^{2+} oscillations induced by hormonal stimulation of individual fura-2-loaded hepatocytes. J Biol Chem 1989；264：12859−12866.
23. Blackmore PF, Waynick LE, Blackman GE, Graham CW, Sherry RS：α-and β-adrenergic stimulation of parenchymal cell Ca^{2+} influx. J Biol Chem 1984；259：12322−12325.
24. Hughes BP, Milton SE, Barrit GJ, Auld AM：Studies with verapamil and nifedipine provide evidence for the presence in the liver cell plasma membrane of two types of Ca^{2+} inflow transporter which are dissimilar to potential-operated Ca^{2+} channels. Biochem Pharmacol 1986；35：3045−3052.
25. Marchetti C, Premont RT, Brown AM：A whole-cell and single-channel study of the voltage-dependent outward potassium current in avian hepatocytes. J Gen Physiol 1988；91：255−274.
26. Sawanobori T, Takanashi H, Hiraoka M, Iida Y, Kamisaka K, Maezawa H：Electrophysiological properties of isolated rat liver cells. J Cell Physiol 1989；139：580−585.
27. Kuno M, Gardner P：Ion channels activated by inositol 1, 4, 5-trisphosphate in plasma membrane of human T-lymphocytes. Nature 1987；326：301−304.
28. Lückhoff A, Clapham DE：Inositol 1, 3, 4, 5-tetrakisphosphate activates an endothelial Ca^{2+} permeable channel. Nature 1992；355：356−358.
29. Putney JW Jr, Bird G StJ：The signal for capacitative calcium entry. Cell 1993；75：199−201.
30. Randriamampita C, Roger YT：Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. Nature 1993；364：809−814.
31. Hansen CA, Yang L, Williamson JR：Mechanisms of receptor-mediated Ca^{2+} signaling in rat hepatocytes. J Biol Chem 1991；266：18573−18579.
32. Striggow F, Bohnensach R：Inositol 1, 4, 5-trisphosphate activates receptor-mediated calcium entry by two different pathways in hepatocytes. Eur J Biochem 1994；222：229−234.

（Received, November 18, 1998）
（Accepted for publication, January 11, 1999）