《Review》

Functional Ca^{2+}-couplings among the mitochondrion, endoplasmic reticulum and plasmalemma in thermogenic brown adipocytes: Possible roles in energy consumption

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Abstract

Obesity is the result of excess energy intake and/or decreased energy consumption. Energy is consumed by basal metabolic activity, muscle activity and thermogenesis. Adrenergic activations of lipolysis and uncoupling proteins in brown adipocytes lead to heat production without oxidative phosphorylation. The energy dissipated by this process is the H^+ electrochemical potential across the mitochondrial membrane generated by H^+ pumps, respiratory chains, that require NADH and $FADH_2$ produced by Ca^{2+}-dependent dehydrogenases in TCA cycle. Thus, this process is strongly affected by the level of intracellular free Ca^{2+} ($[Ca^{2+}]_i$), which are regulated by Ca^{2+} binding to Ca^{2+} binding proteins, Ca^{2+} entry and extrusion at the plasma membrane, Ca^{2+} release and uptake into, and from, mitochondria and the endoplasmic reticulum (ER). We review here how these organelles and the plasmalemma communicate with each other in regulating $[Ca^{2+}]_i$ and discuss how these coupling are involved in thermogenesis in rat brown adipocytes. Our recent observations suggest the new mechanisms of $[Ca^{2+}]_i$ regulation in brown adipocytes: 1) uncoupling of oxidative phosphorylation and the subsequent re-coupling activate Ca^{2+} entry at the plasmalemma that depends on restoration of H^+ electrochemical potential or ATP synthesis, 2) mitochondrial Ca^{2+} release induces Ca^{2+} release from the ER, 3) Ca^{2+} depletion in the ER via mitochondria-induced Ca^{2+} release activates store-operated Ca^{2+} entry (SOC) in a fraction of cells and 4) Ca^{2+} depletion in the ER activates Ca^{2+} release from mitochondria. Since these mechanisms are activated by the α- and β-actions of noradrenaline, they likely play important roles in thermogenesis.

Obesity that leads to a variety of diseases results from the imbalance of energy uptake and consumption. Excess energy intake and/or decreased energy consumption are caused by impairment of hypothalamic appetite control, metabolic abnormalities, decreased muscle activity and reduction of nonshivering thermogenesis. Brown adipose tissue is a thermogenic organ that dissipates energy in response to cold exposure (1, 2). The β-action of noradrenaline released by sympathetic activity causes lipolysis and enhances production of NADH and $FADH_2$ via activation of TCA cycle in mitochondria. Free fatty acids produced activates uncoupling proteins in mitochondria, which shunts H^+ electrochemical potential at the inner membrane, generating heat without

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oxidative phosphorylation (3-5). For this process to occur, activation of Ca^{2+}-dependent dehydrogenases is needed to produce NADH and $FADH_2$ (6, 7). Thus, increases in free Ca^{2+} concentration in mitochondria is the absolute requirement for thermogenesis and so energy consumption.

In brown adipocytes, $[Ca^{2+}]_i$ is largely increased by the release of Ca^{2+} from the ER through IP_3 receptors (8-11) and the subsequent activation of store-operated Ca^{2+} entry (SOC:12, 13) in response to the α-action of noradrenaline. This α-action could obviously be complementary to the β-action generating heat. Then, it is possible that these functions of mitochondria and the ER interact with each other via Ca^{2+} dynamics. We review here how these organelles and the plasma membrane communicate with each other in regulating intracellular Ca^{2+} based on our recent experimental findings and discuss how these couplings are involved in thermogenesis in rat brown adipocytes. Several lines of evidence suggest the new mechanisms of $[Ca^{2+}]_i$ regulation in brown adipocytes (14). First, uncoupling of oxidative phosphorylation in mitochondria and the subsequent re-coupling activate a new mode of plasmalemmal Ca^{2+} entry that depends on restoration of H^+ electrochemical potential or ATP synthesis. Second, Ca^{2+} release from mitochondria causes Ca^{2+} release from the ER and the resultant Ca^{2+} depletion in the ER activates SOC in a fraction of cells. Third, Ca^{2+} release from the ER results in depletion of Ca^{2+} in mitochondria as well as activation of SOC. Since these mechanisms are activated by the actions of noradrenaline and if one considers the indispensability of intracellular Ca^{2+} to build up the H^+ electrochemical potential (μ_H) across the mitochondrial membrane, the driving force for heat induction, they likely play important roles in thermogenesis.

Thermogenesis in brown adipocytes

Brown adipose tissues are located mainly in interscapular regions and fragmentally scattered in other body areas of rodents and infants of human. They are heavily supplied with blood vessels and dense sympathetic innervation. Brown adipocytes are endowed with numerous mitochondria and fragmented lipid droplets, but not a single large lipid drop (1, 15). Dense blood supplies and numerous mitochondria render the brown adipose tissue brownish appearance. Exposure to cold environment enhances the activity of neurons in the hypothalamus. This activates sympathetic nervous systems via the midbrain and eventually leads to the release of noradrenaline toward brown adipocytes (16-18). The activation of β_3-adrenergic receptor on the cell membrane of brown adipocyte enhances hydrolysis of neutral lipids, which leads to production of NADH and $FADH_2$ via activation of Ca^{2+}-dependent dehydrogenases. Free fatty acids produced by lipolysis activate the type 1 uncoupling protein (2, 19) that

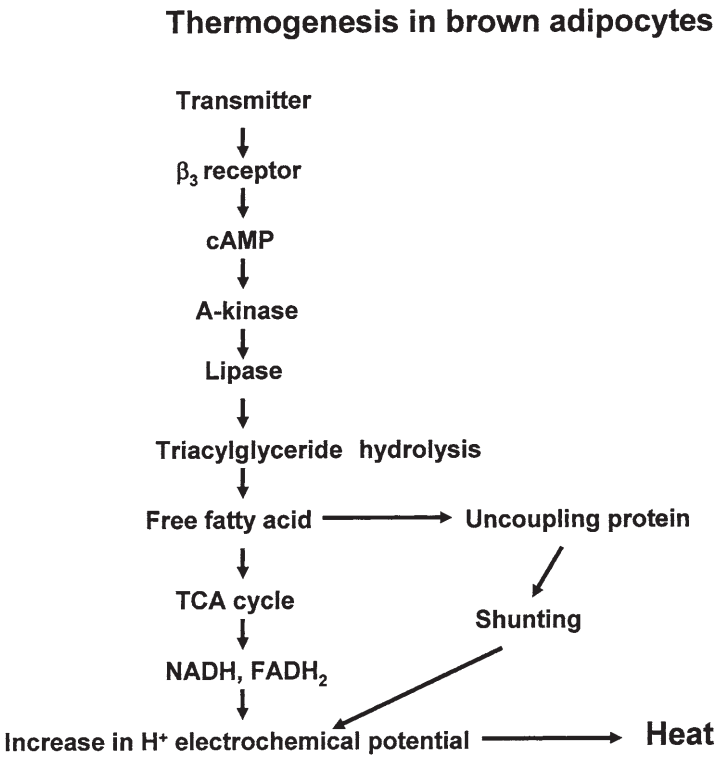

Fig. 1. The mechanisms of thermogenesis in brown adipocytes. See text.

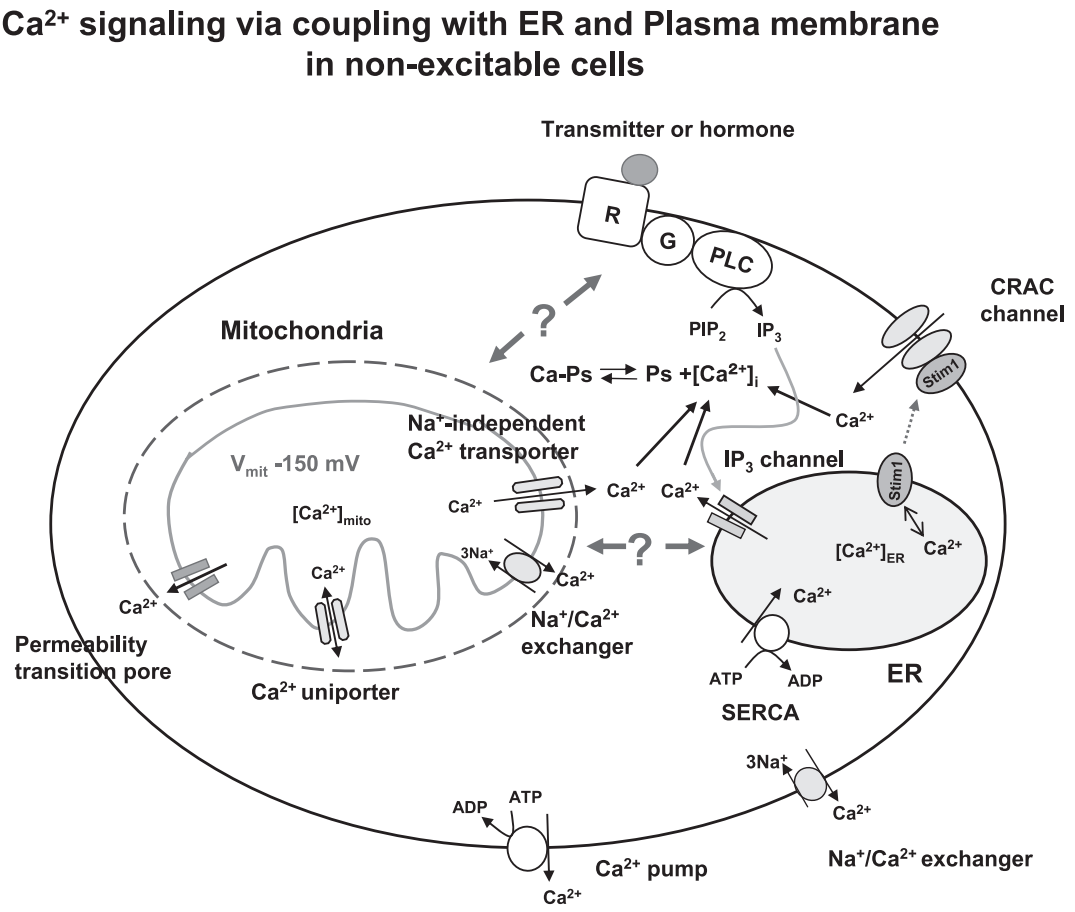

Fig. 2. Ca^{2+} signaling in brown adipocytes. Ps is Ca^{2+}-binding proteins, PIP_2 phosphatidylinositol 4,5-bisphosphate, PLC Phospholipase C, G GTP-binidng protein, R receptor for neurotransmitter or hormone, SERCA sarcoplasmic/endoplasmic Ca^{2+} pump. CRAC Ca^{2+}-release activated cation channel is one of the channels involved in SOC. Stim1 is a protein that normally sits in the membrane of the ER and binds with Ca^{2+}. When the free Ca^{2+} level is decreased in the lumen of the ER, Stim1 migrates to the plasmalemma and activates SOC. Permeability transition pore in the inner mitochondrial membrane is a large conductance channel that allows to pass many species of ions and opens usually when apoptosis starts to take place. Presumably it also operates in response to various conditional changes under the physiological conditions. See text.

shuttles H^+ across the mitochondrial inner membrane, shunting μ_H that is increased by enhanced production of NADH and $FADH_2$ (3, 4: Fig. 1). On the other hand, α_1-activation of IP_3 receptors by noradrenaline causes release of Ca^{2+} from the ER (8-11). The resultant depletion of Ca^{2+} in the ER activates SOC (13: Fig. 2). These increases in $[Ca^{2+}]_i$ by the α-actions would also contribute to thermogenesis.

Regulation and functions of intracellular free Ca^{2+} in general

To facilitate the understanding of this review by the readers outside the fields of physiology and cell biology, how $[Ca^{2+}]_i$ is regulated and what functions it exerts in general are described in some details (Fig. 2). Free Ca^{2+} concentration in the cytosol is normally set at less than 100 nM, one ten thousandth of the extracellular Ca^{2+}, while a large amount of Ca^{2+} is bound to Ca^{2+}-binding proteins and stored in Ca^{2+}-storing organelles (Ca^{2+}-stores), such as ER and mitochondria.

The extremely low level of $[Ca^{2+}]_i$, which is prerequisite for intracellular Ca^{2+} signaling for various cellular functions, is achieved by powerful Ca^{2+} extrusion at the plasma membrane via activity of Ca^{2+} pumps and Na^+/Ca^{2+} exchangers (20, 21: Fig. 1). Ca^{2+} that is released from Ca^{2+}-stores or enters at the plasmalemma into the cytosol is buffered by binding to Ca^{2+}-binding proteins, Ca^{2+} extrusion at the cell membrane and Ca^{2+} uptake

into Ca^{2+}-stores so that a rise in $[Ca^{2+}]_i$ became slower in time course and smaller in amplitude. The membrane of the ER and sarcoplasmic reticulum (SR) are endowed with sarcoplasmic/endoplasmic Ca^{2+} pumps (SERCA) for taking up Ca^{2+} into the lumen and also with Ca^{2+} release channels, IP_3 receptors or ryanodine receptors for releasing Ca^{2+} into the cytosol. The former channel is activated by IP_3 and/or Ca^{2+}, while the latter is by Ca^{2+} and/or the electrical coupling with voltage-gated Ca^{2+} channel at the plasma membrane. In the lumen of the ER and SR, Ca^{2+} is largely bound by Ca^{2+}-binding proteins enabling a large amount of Ca^{2+} stored. Thus, the ER and also SR are the important source and store of Ca^{2+} for Ca^{2+} signaling (Fig. 2).

Mitochondria is also the important Ca^{2+} source and store. Mitochondria take up Ca^{2+} into the lumen through a Ca^{2+} uniporter driven by the large inside-negative μ_H (22-24). The uniporter is now identified as a Ca^{2+} permeable channel (25). Mitochondria releases Ca^{2+} through Na^+/Ca^{2+} exchangers, Na^+-independent Ca^{2+} transporters, permeability transition pores and even Ca^{2+} uniportetrs, once the $[Ca^{2+}]_i$ became lower than the threshold for Ca^{2+} uptake (Fig. 2). In the matrix of mitochondria, Ca^{2+} is largely bound to phosphates so that acidification raises the free Ca^{2+} concentration in the matrix. Furthermore, recent studies suggest that the intimate interaction of mitochondria with the ER maintains the activation of SOC by releasing Ca^{2+} from the ER (26-31).

A rise in $[Ca^{2+}]_i$ via Ca^{2+} entry through voltage-gated or transmitter-operated Ca^{2+} channels and/or Ca^{2+} release from the sarcoplasmic or ER causes a variety of functions in excitable cell: muscle contraction, the release of neurotransmitter, the modulation of synaptic transmission, the formation of memory, gene activation, growth and others in neurons (31-34). Rises in $[Ca^{2+}]_i$ by Ca^{2+} release from the ER via inositol 1,4,5-trisphosphate (IP_3) receptors (35) and Ca^{2+} entry through the plasma membrane as a result of depletion of Ca^{2+} in the ER (SOC:12, 36: Fig. 1) results in many functions of nonexcitable cells , including cell growth, exo- and endocytosis, gene expression, malignant formation and others.

Ca^{2+} coupling of mitochondria to the ER in brown adipocytes

Functional coupling of mitochondria to the ER in respect to Ca^{2+} dynamics can be revealed by uncoupling of oxidative phosphorylation in mitochondria by the action of a protonophore, carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (FCCP). FCCP (2 μM) applied for 3 min produces the di- or tri-phasic rises in $[Ca^{2+}]_i$ (Fig. 3A and B). Similar di- or tri-phasic rises were induced by another uncoupler, dinitrophenol, (DNP), or a blocker of electron transport, rotenone. The first quickly-rising transient phase of FCCP-induced rises is accompanied by the depolarization of mitochondrial membrane and is not affected by a Ca^{2+}-free, EGTA solution with or without EGTA (Fig. 3B) and in a Na^+ deficient or free solution. Thus, mitochondrial depolarization caused by FCCP releases Ca^{2+} from mitochondria under the increased concentration of free Ca^{2+} in the matrix by acidification, constituting the initial phase of FCCP-induced rises. Since it is not affected by applying a blocker of Ca^{2+} uniporter, ruthenium red, an inhibitor of permeability transition pores, cyclosporine A (37), and a Na^+ free solution, which inhibits Na^+/Ca^{2+} exchanger, Ca^{2+} release occurs through a Na^+-independent Ca^{2+} pathway at the mitochondrial membrane.

The second spiky rise in $[Ca^{2+}]_i$ is partially abolished in a nominally Ca^{2+} free or Ca^{2+} free, EGTA solution, leaving a small external Ca^{2+}-independent component (Fig. 3B). Thus, the second phase of FCCP-induced rises in $[Ca^{2+}]_i$ consists of two components; one is external Ca^{2+} dependent, while another is independent of extracellular Ca^{2+} (Fig. 4). Accordingly, the uncoupling of oxidative phosphorylation that causes depolarization and the resultant Ca^{2+} release from mitochondria activate a transient Ca^{2+} entry at the plasmalemma and Ca^{2+} release from a Ca^{2+} store other than mitochondria. Aside from the mechanism of Ca^{2+} entry, which is described

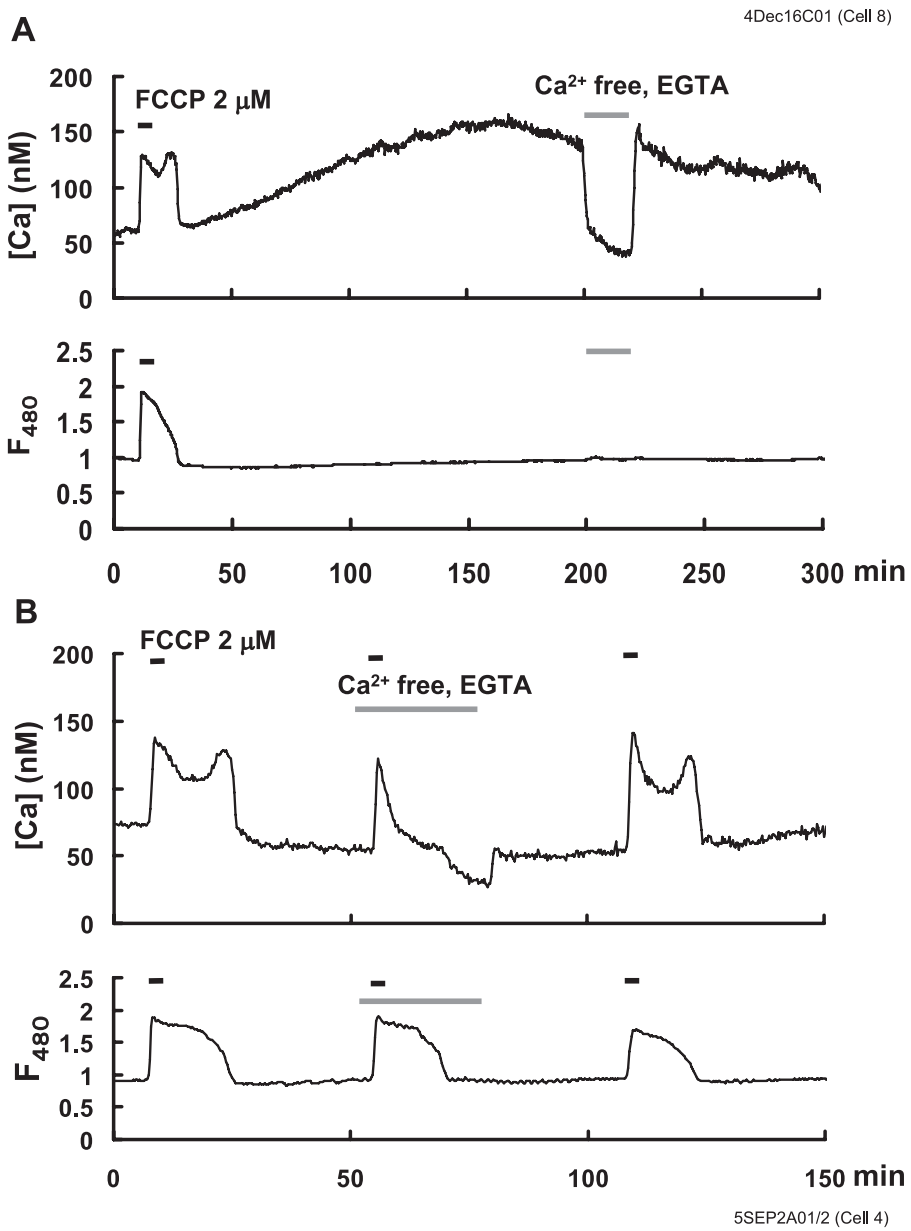

Fig. 3. Rises in $[Ca^{2+}]_i$ and mitochondrial membrane depolarization induced by FCCP and effects of a Ca^{2+} free, EGTA solution. *A*: Triphasic rises in $[Ca^{2+}]_i$ with mitochondrial membrane depolarization and effects of a Ca^{2+} free, EGTA solution on the third phase of rises. The lower graph indicates the time course of changes in fluorescence of rhodamine 123 (F_{480}) that reflects a change in mitochondrial membrane potential. An increase in F_{480} implies a decrease in membrane potential. A solution containing FCCP (2 μM) or a Ca^{2+} free, EGTA solution was superfused to the bath during a period indicated by a black or gray horizontal bar, respectively. *B*: Diphasic rises in $[Ca^{2+}]_i$ with membrane depolarization and effects of a Ca^{2+} free, EGTA solution. Explanations are similar to those in *A*. Reproduced from Kuba et al. (14) by permission of Am. J. physiol. **Methods:** Rats (Wistar ST or WKAH) kept at 4°C for 5 to 7 hours were killed, the procedures approved by Animals Ethics Committee of Nagoya University (Nagoya, Japan). Brown adipocytes were isolated and cultured for 2 to 7 days (34). Adipocytes were loaded with fura-2/AM (5 μM) or Oregon Green 488 BAPTA-1/AM (OGB-1: 5 μM) . Changes in $[Ca^{2+}]_i$ was measured with conventional ratio-imaging systems using fura-2 in most experiments or single wavelength-imaging using Oregon Green 488 BAPTA-1 in some experiments. Changes in mitochondrial membrane potential were measured by recording changes in fluorescence of rhodamine 123 loaded together with fura-2/AM.

in detail below, Ca^{2+} release caused by mitochondrial uncoupling appears to take place from the ER. If so, it is expected that once the ER is depleted of Ca^{2+} by inhibiting Ca^{2+} uptake, the Ca^{2+} release-dependent component of the second phase would disappear. This is indeed the case. The application of cyclopiazonic acid (CPA: 10 μM), a blocker of the SERCA, eliminated this Ca^{2+} release component with the marked depression of the first

component (for Ca^{2+} release from mitochondria activated by Ca^{2+} release from the ER: see below for the mechanism: Fig. 5A). Similar abolition occurs after the application of an irreversible blocker of SERCA, thapsigargin (Fig. 6). Alternatively, the CPA-induced rise in $[Ca^{2+}]_i$ in a Ca^{2+} free solution was markedly decreased after the application of FCCP (Fig. 5B). Thus, it is of no doubt that Ca^{2+} release from the ER is involved in the second phase. Ca^{2+} release from the ER by the Ca^{2+} liberated from mitochondria would occur via a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism through IP_3 receptors or ryanodine receptors (35, 38, 39). The former is the case, as shown below.

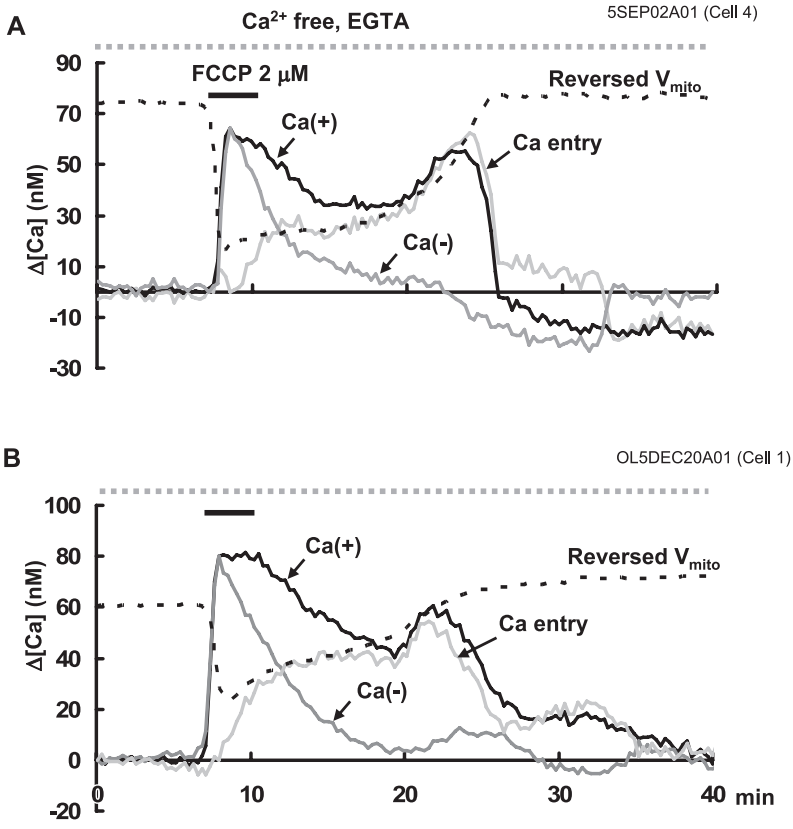

Fig. 4. Comparison of the time courses of the Ca^{2+} entry-dependent component of the second spiky rise in $[Ca^{2+}]_i$ and mitochondrial depolarization induced by FCCP. The Ca^{2+} entry-dependent component of the second spiky rise (light gray trace) was isolated by subtracting the FCCP-induced response in a Ca^{2+} free, EGTA solution (dark gray trace) from that in Krebs solution (black trace). Changes in mitochondrial membrane potential was shown in the reversed direction (interrupted trace) and superimposed so that a repolarizing phase can be compared with the time course of the Ca^{2+} entry-dependent component of the second spiky rise. FCCP (2 μM) was applied for 3 min as shown by a black horizontal bar. The panels in *A* and *B* are the analyses from two different cells. Reproduced from Kuba et al. (14) by permission of Am. J. physiol.

The involvement of PLC in the activation of Ca^{2+} release from the ER by mitochondrial Ca^{2+} release can be assessed by the action of a blocker of phospholipase C (PLC), U-73122. In the presence of the blocker, no second component of FCCP-induced rises is seen in a Ca^{2+} free solution (Fig. 7A and B). The blocking action of U-73122 on the second component is seen even in the presence of external Ca^{2+} as the depression of the "valley" between the first and second phases of FCCP-induced rises in $[Ca^{2+}]_i$ (Fig. 7C and D).

Coupling of mitochondrial activity to two modes of Ca^{2+} entry at the plasma membrane

One mode of Ca^{2+} entry activated by mitochondrial activity is the activation of SOC in response to Ca^{2+} depletion in the ER as the result of mitochondria-induced CICR. This produces the late slow rise of FCCP-induced rises in $[Ca^{2+}]_i$ in some fractions of cells as evidenced by its marked reduction in a Ca^{2+} free solution (Fig. 3A). Another mode of Ca^{2+} entry induced by mitochondrial activity is reflected in the external Ca^{2+}-dependent component of the second phase of FCCP-induced rises in $[Ca^{2+}]_i$. The mechanism of this Ca^{2+} entry is likely to involve a process or a product related to restoration of μ_H or ATP synthesis at the mitochondrial membrane after the subsidence of H^+ shunt for the three lines of evidence. First, the rising phase of this Ca^{2+} entry is closely associated with the time course of mitochondrial repolarization after washout of FCCP (Fig. 4). Second, this Ca^{2+} entry component is accompanied by a rise in intracellular Mg^{2+} concentration (Fig. 7A). This

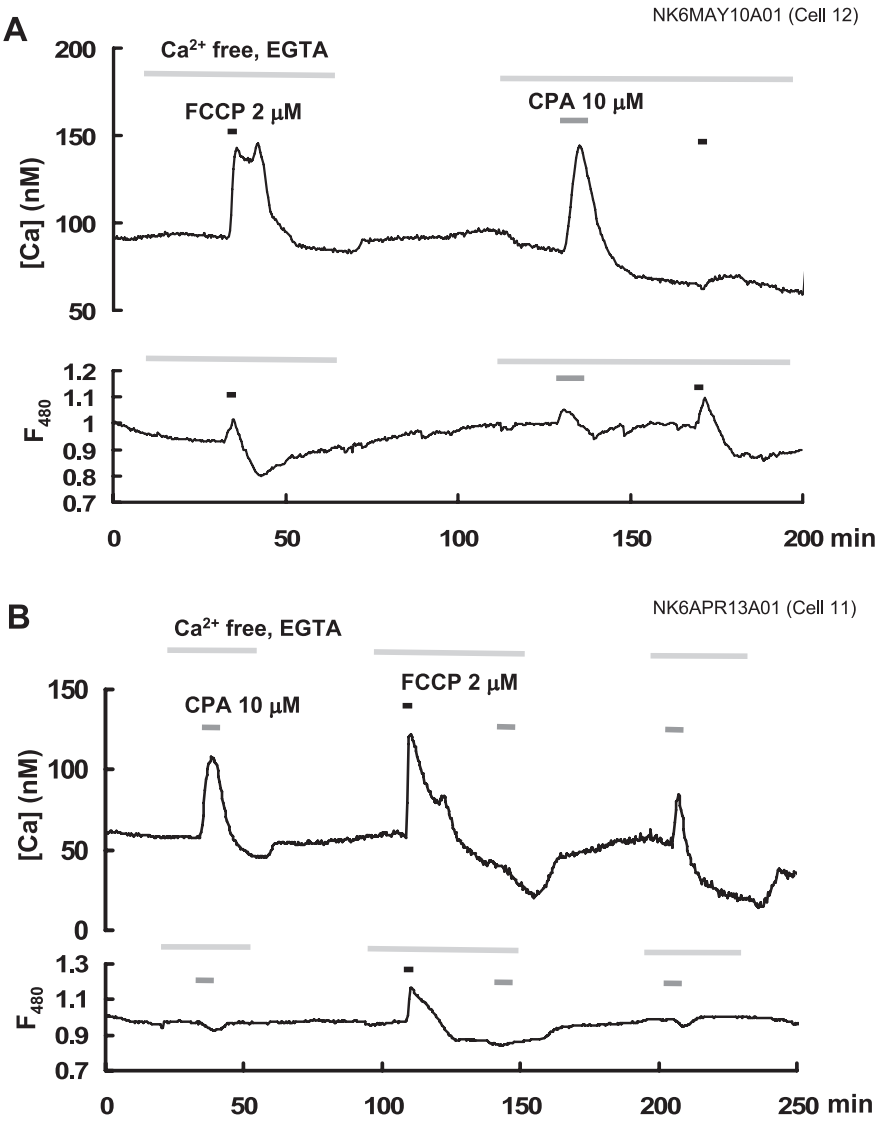

Fig. 5. Effects of the prior application of CPA on FCCP-induced rises in $[Ca^{2+}]_i$ in a Ca^{2+} free, EGTA solution and effects of prior application of FCCP on CPA-induced rises in $[Ca^{2+}]_i$ in a Ca^{2+} free, EGTA solution. *A*: Effects of the preceding application of CPA on FCCP-induced rises in $[Ca^{2+}]_i$ in a Ca^{2+} free, EGTA solution. FCCP (2 μM) was applied for 3 min as shown by a black horizontal bar. A Ca^{2+} free, EGTA solution was applied for the period indicated by a light gray bar, while CPA (10 μM) was for 8 min as indicated by a dark gray bar. The lower trace showing relative changes in F_{480} to the initial fluorescence reflects those in mitochondrial membrane potential. *B*: Effects of the preceding application of FCCP on CPA-induced rises in $[Ca^{2+}]_i$ in a Ca^{2+} free, EGTA solution. Explanations are similar to those in *A*. Reproduced from Kuba et al. (14) by permission of Am. J. physiol.

implies hydrolysis of ATP, which releases Mg^{2+} from Mg^{2+}-ATP. Third, the second phase of FCCP-induced rise is blocked by a blocker of ATP synthase (Fig. 8B). This mode of Ca^{2+} entry, tentatively called μ_H-dependent Ca^{2+} entry, however, does not take place through a channel involved in SOC, because it is not affected by Na^+ deficient solution, while SOC is markedly reduced. It is to be noted that either mode of Ca^{2+} entry is not caused by the inhibition of Ca^{2+} extrusion via activity of Na^+/Ca^{2+} exchangers at the plasma membrane, since the μ_H-dependent Ca^{2+} entry is generated in a Na^+ free solution, while SOC is markedly depressed by the removal of external Na^+. Furthermore, both the modes are not the result of the inhibition of Ca^{2+} pump at the plasmalemma. Raising the external pH did not affect the μ_H-dependent Ca^{2+} entry, but it enhanced SOC.

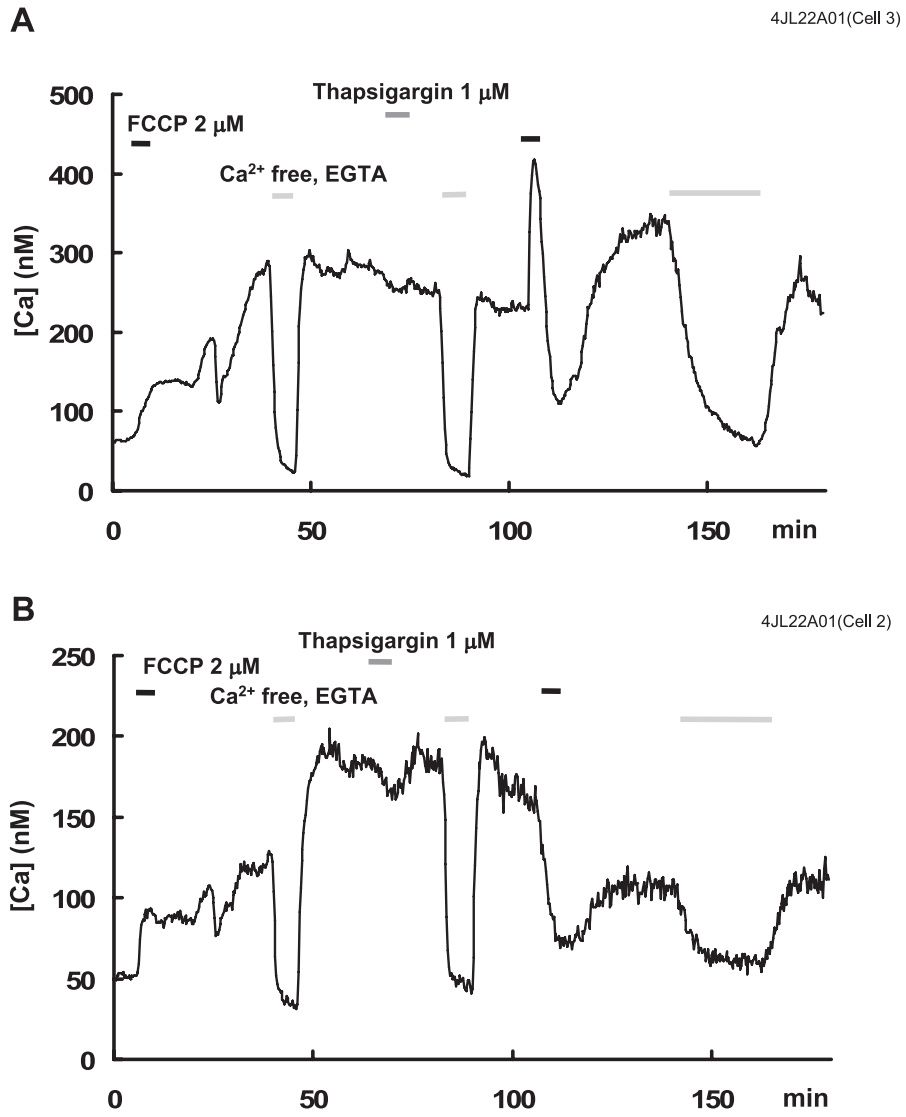

Fig. 6. Functional Ca^{2+}-couplings between mitochondria and the ER. *A*: A Ca^{2+}-coupling of mitochondria to the ER. FCCP produces triphasic rises in $[Ca^{2+}]_i$. Thapsigargin had no effects on $[Ca^{2+}]_i$ during the third phase of rises in $[Ca^{2+}]_i$, while the third phase was completely abolished by the removal of the external Ca^{2+}. FCCP (2 μM) and thapsigargin (1 μM) were applied during the period indicated by a black and dark gray bar, respectively. A Ca^{2+} free, EGTA solution was superfused to the bath during a period indicated by a light gray bar. *B*: Bidirectional Ca^{2+}-couplings between mitochondria and the ER. FCCP produces triphasic rises in $[Ca^{2+}]_i$. Thapsigargin had again no effects on $[Ca^{2+}]_i$ during the third phase of FCCP-induced rises in $[Ca^{2+}]_i$, while the third phase was completely abolished by the removal of the external Ca^{2+} and transiently by the second application of FCCP. Other explanations are the same as those in *A*. Reproduced from Kuba et al. (14) by permission of Am. J. physiol.

Ca^{2+} coupling of the ER to mitochondria

Ca^{2+} release from the ER activates mitochondrial Ca^{2+} release or uptake. FCCP (2 μM) applied during the sustained rise in $[Ca^{2+}]_i$ by thapsigargin produces two types of actions depending on the cell. In a fraction of cell, Ca^{2+} uptake takes place in response to Ca^{2+} release from the ER, as evidenced by a sharp rise in $[Ca^{2+}]_i$ during the sustained rise by blocking SERCA at the ER membrane. The extent of Ca^{2+} uptake into mitochondria, however, varies among cells. The greater Ca^{2+} uptake occurs for the increased level of $[Ca^{2+}]_i$ under the effect of thapsigargin in a fraction of cells (Figs. 6A and 9B), while the smaller Ca^{2+} uptake takes place even at a high level of $[Ca^{2+}]_i$ in other fraction of cells. This implies that a fraction of Ca^{2+} stored in mitochondria is released in this type of cells. In another type of cells, complete release of Ca^{2+} from mitochondria

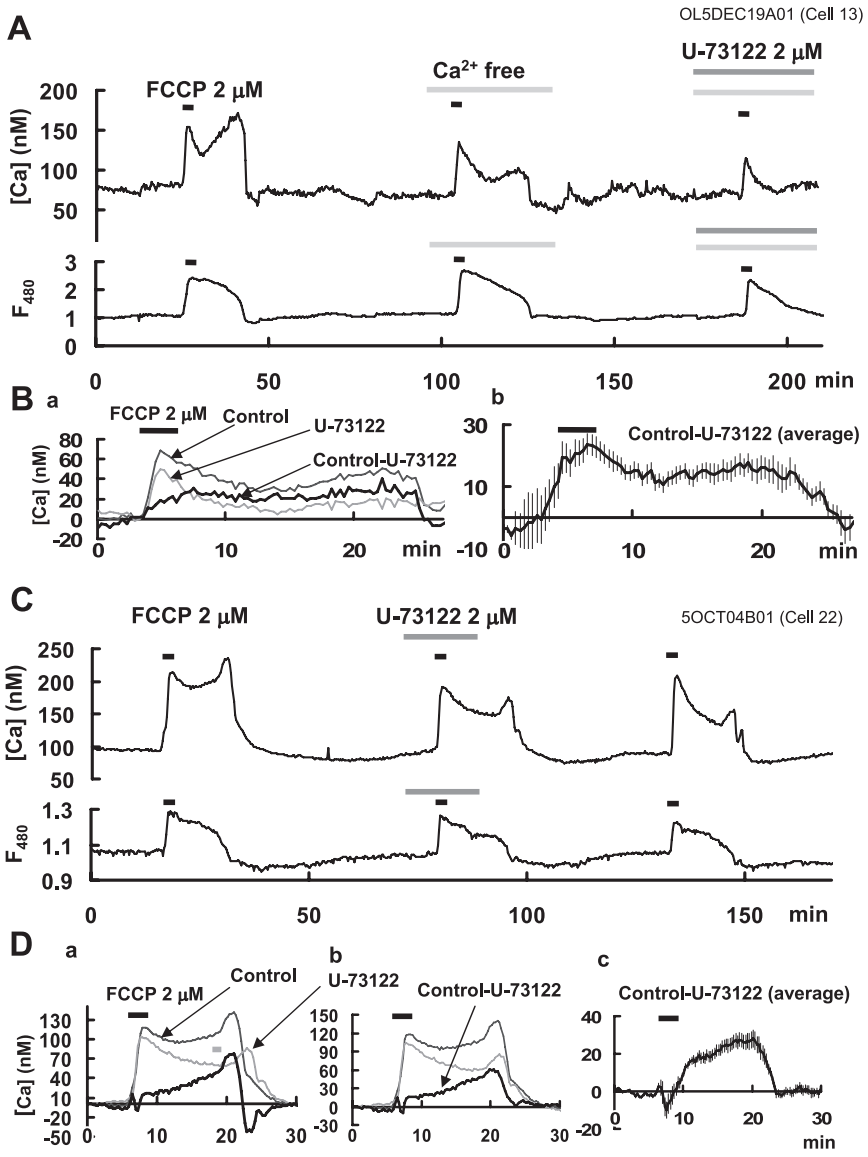

Fig. 7. Effects of a blocker of PLC on FCCP-induced rises in $[Ca^{2+}]_i$ and associated changes in mitochondrial membrane potential in Krebs and Ca^{2+} free, EGTA solutions. *A*: Effects of U-73122 (2 μM) on FCCP-induced rises in $[Ca^{2+}]_i$ and mitochondrial membrane potential changes in a Ca^{2+} free, EGTA solution. FCCP (2 μM) was applied for 3 min as shown by a black horizontal bar. A solution containing U-73122 (2 μM) was applied for the period indicated by a dark gray bar, a Ca^{2+} free, EGTA solution was applied for the period indicated by a light gray bar. *Ba*: Superposed traces of FCCP-induced rises in $[Ca^{2+}]_i$ in the absence and presence of U-73122 (2 μM) and the U-73122-sensitive component in a Ca^{2+} free solution shown in *A*. *Bb*: Averaged U-73122-sensitive component (mean ± SE of mean) obtained from 10 cells. *C*: Effects of U-73122 (2 μM) on FCCP-induced rises in $[Ca^{2+}]_i$ and mitochondrial membrane potential changes in Krebs solution. Explanations are the same as those in *A* except that U-73122 was applied in Krebs solution. *Da*: Superposed traces of FCCP-induced rises in $[Ca^{2+}]_i$ in the absence and presence of U-73122 (2 μM) and the U-73122-sensitive component of FCCP-induced response in Krebs solution shown in *C*. *Db*: Superposed traces of control responses and that in the presence of U-73122 (2 μM) and the U-73122-sensitive component in Krebs solution shown in *C*. A part of the trace in the presence of U-73122 indicated by a light gray bar in *Da* was deleted to eliminate the delay in the onset of the second component of FCCP-induced rises. *Dc*: Averaged U-73122-sensitive component (mean ± SE of mean) obtained from 13 cells, in which a part of the trace in the presence of U-73122 was deleted. Reproduced from Kuba et al. (14) by permission of Am. J. physiol.

occurs in response to the Ca^{2+} released from the ER via CICR (Figs. 6B and 9A). The Ca^{2+} release channel involved in CICR at the mitochondrial membrane in brown adipocytes, however, is not yet identified, although Ca^{2+} uniporters or permeability transition pores are suggested in other cells (24, 40).

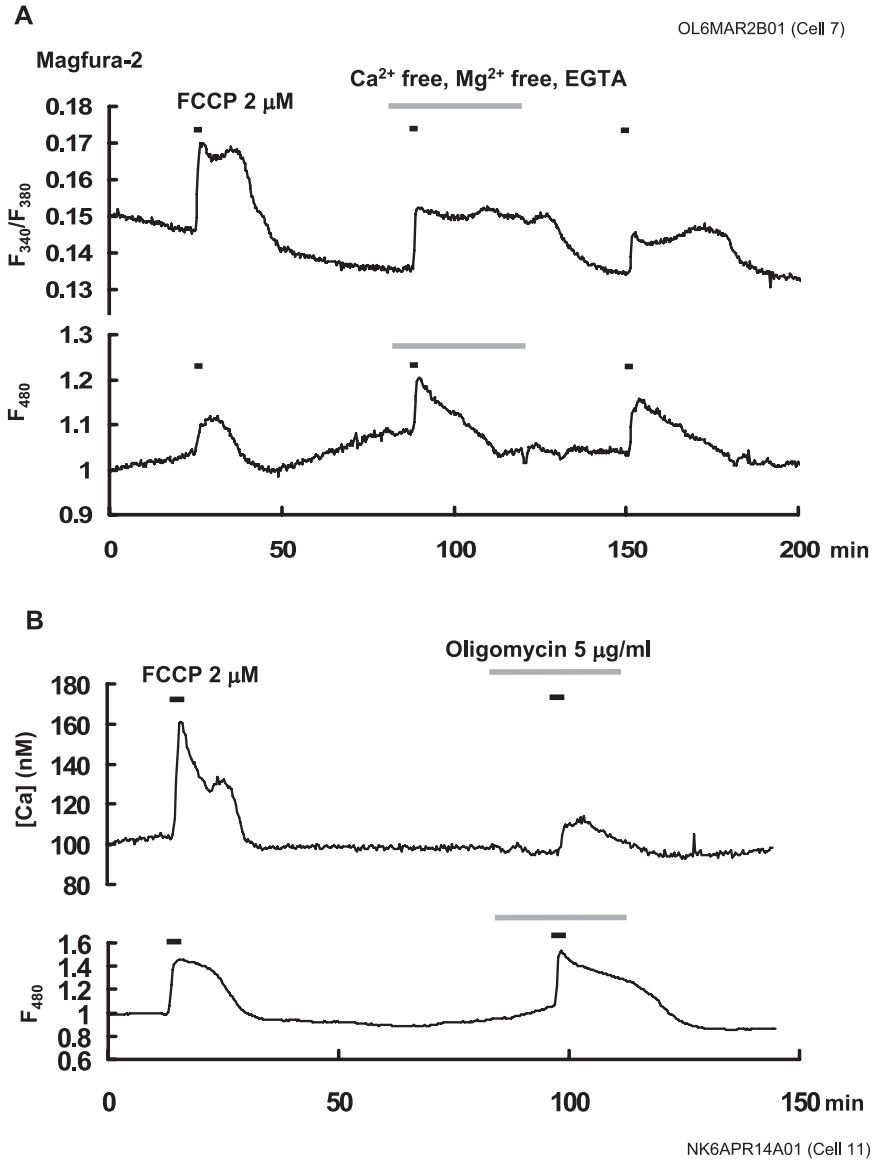

Fig. 8. Effects of a Ca^{2+} free, Mg^{2+} free, EGTA solution on FCCP-induced changes in Magfura-2 fluorescence ratio and effects of oligomycin on FCCP-induced rises in $[Ca^{2+}]_i$ and mitochondrial membrane depolarization. *A*: Effects of a Ca^{2+} free, Mg^{2+} free, EGTA solution on the FCCP-induced changes in the ratio of Magfura-2 fluorescence. The ratio of fluorescence at 340 nm and 380 nm was taken. FCCP (2 μM) was applied for 3 min as shown by a black bar, while a Ca^{2+} free, Mg^{2+} free, EGTA solution was applied for the period indicated by a gray bar. Magfura-2 was loaded and ratio-imaging was made. *B*: Effects of oligomycin on FCCP-induced rises in $[Ca^{2+}]_i$ and mitochondrial membrane depolarization. FCCP (2 μM) was applied for 4 min as shown by a black horizontal bar, while a solution containing oligomycin (5 μg/ml) was applied for the period indicated by a gray bar. The lower trace (F_{480}) represents changes in mitochondrial membrane potential. Reproduced from Kuba et al. (14) by permission of Am. J. physiol.

Possible roles of mitochondrion-ER-plasmalemma couplings in thermogenesis

The central questions are how the couplings among the mitochondrion, ER and plasmalemma operate under the physiological conditions and how they affect or regulate heat production in brown adipocytes. Clues to answer these questions should lie in the modes of the β-action of noradrenaline involved in thermogenesis, and must be obtained by observing changes in heat production under the blockade of each of these couplings. In addition, it is highly possible that the α-action of noradrenaline plays a crucial role in thermogenesis, although it is overlooked for a long time.

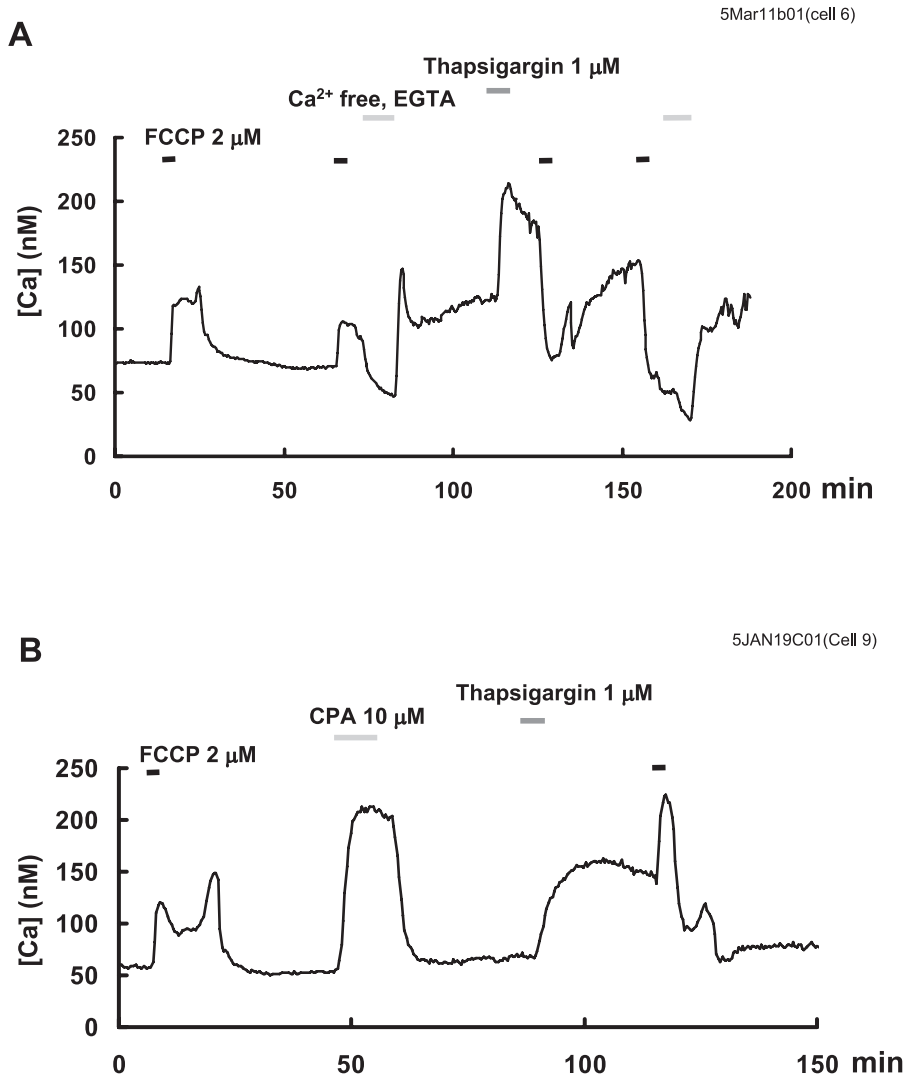

Fig. 9. Effects of FCCP on $[Ca^{2+}]_i$ during the sustained rise in $[Ca^{2+}]_i$ induced by thapsigargin. *A*: Reduction of $[Ca^{2+}]_i$ by FCCP (2 μM) during the thapsigargin-induced rise in $[Ca^{2+}]_i$. The record also shows that the second spiky component of rises remains during the FCCP-induced reduction of $[Ca^{2+}]_i$, but is blocked in Ca^{2+} free, EGTA solution. FCCP and Thapsigargin was applied during the period indicated by a black or dark gray bar, respectively. A Ca^{2+} free, EGTA solution was superfused to the bath during the period indicated by a light gray bar. *B*: Diphasic rises induced by FCCP during the sustained rise in $[Ca^{2+}]_i$ caused by thapsigargin. The record also shows that the reduction of $[Ca^{2+}]_i$ following the initial transient rise underlies the second spiky rise. FCCP and thapsigargin were applied during the period indicated by a black and dark gray bar, respectively. CPA (10 μM) was applied during the period indicated by a light gray bar simply to examine the potency of thapsigargin to deplete Ca^{2+} in the ER. Reproduced from Kuba et al. (14) by permission of Am. J. physiol.

Under the physiological conditions, the actions of FCCP shown above is reproduced by the activation of uncoupling proteins by the β-action of noradrenaline released from sympathetic nerves in response to cold exposure (see the previous section for references). On the other hand, the action of CPA and thapsigargin are reproduced by the α-action of noradrenaline (see the previous section for references). Thus, the two modes of the actions of noradrenaline can produce those induced by FCCP, CPA and thapsigargin.

Isoprenaline, β-agonist, elicits Ca^{2+} release from mitochondria and then activates metabolite-dependent Ca^{2+} entry, Ca^{2+} release via the mitochondrion-ER coupling and subsequently SOC (unpublished observations). Moreover, under the physiological conditions, another mode of β-actions occurs that enhances lipolysis and so the driving force for heat production. The notable characteristic of the β-actions is that it lasts for more than several tens of minutes. Thus, the two modes of the β-actions, increasing the μ_H and shunting it, must be in

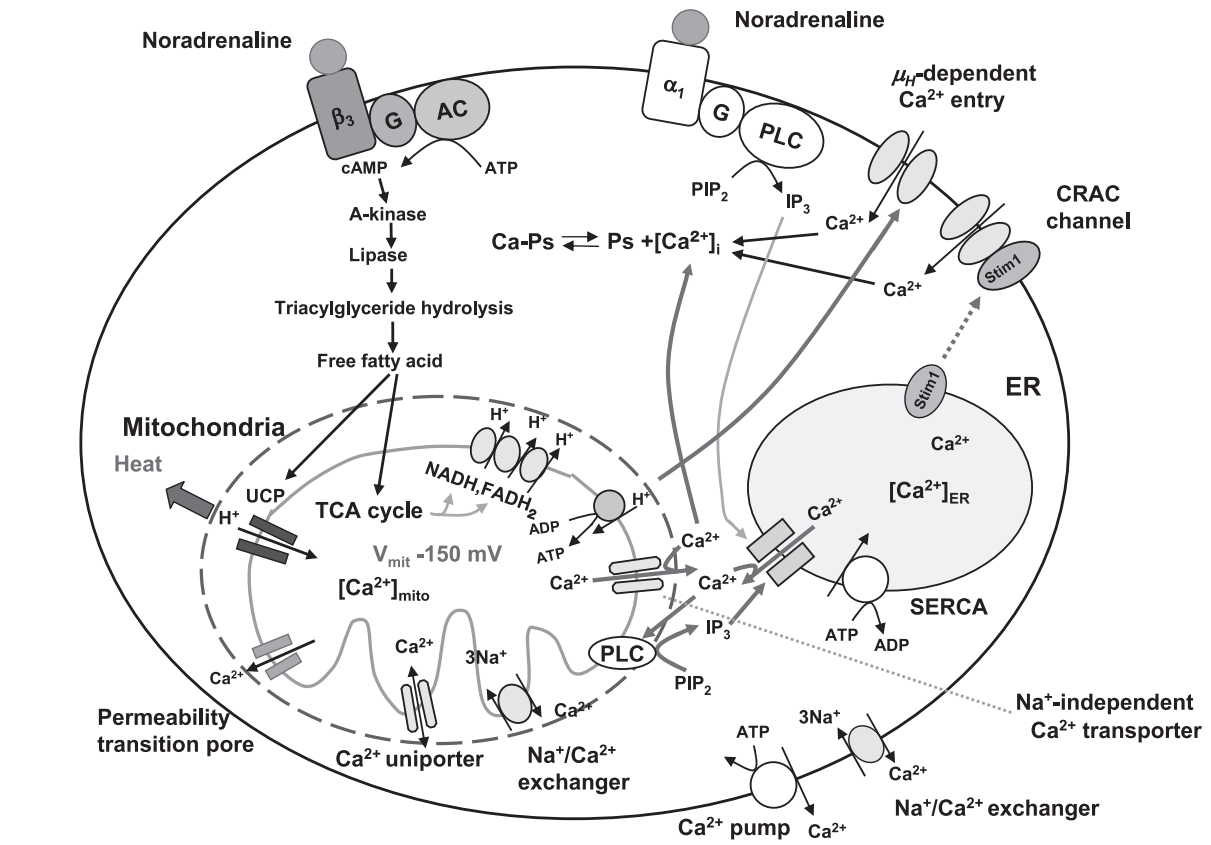

Fig. 10. New mechanisms of Ca^{2+} signaling and possible roles in thermogenesis in brown adipocytes. α_1 is α_1 adrenergic receptor. β_3 is β_3 adrenergic receptor. AC is adenylate cyclase. UCP is the type-1 uncoupling protein. Explanations for other symbols are the same as those in Fig. 2. The dark gray lines show newly found the Ca^{2+} couplings between mitochondria and the ER and those between mitochondria and the plasma membrane. See text.

the steady state during heat genesis. Imagine the situation for a bathtub, whose stopcocks for hot water and sewage are both opened, dumping potential energy and heat without achieving work. To maintain the steady state for energy accumulation and dissipation in mitochondria, a constant production of NADH and $FADH_2$ and their constant oxidation of NADH and $FADH_2$ for pumping H^+ across the inner membrane are needed. The former is achieved by continuous activation of lipolysis and the latter is maintained by the constant high level of $[Ca^{2+}]_i$ that supply Ca^{2+} through Ca^{2+} uniporters for the activation of Ca^{2+}-dpendent dehydrogeneses in mitochondria. This high level of $[Ca^{2+}]_i$ can be maintained by continuous Ca^{2+} entry via the activation of metabolite-dependent Ca^{2+} entry and SOC, which are activated by both the α- and β-actions of noradrenaline via mitochondrion-ER-plasmalemma couplings (Fig. 10).

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