Inositol 1,4,5-Trisphosphate- and Caffeine-Sensitive Ca^{2+} -Storing Organelle in Bovine Adrenal Chromaffin Cells

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ABSTRACT—The uptake and release properties of Ca^{2+} by several subcellular fractions of the bovine adrenal medulla were investigated. Investigation by the $^{45}\text{Ca}^{2+}$ tracer method showed that permeabilized cells and the fractions of mitochondria (MT) and microsomes (MC) caused ATP-dependent Ca^{2+} uptake in a Ca^{2+} concentration-dependent manner (pCa 8–4), whereas permeabilized cells and the fractions of secretory granules (SG) were able to accumulate a significant amount of Ca^{2+} even in the absence of ATP, which was completed by the addition of hexokinase and glucose. In these organelle fractions, Ca^{2+} uptake in the presence of ATP at pCa 7 and pCa 5.8 was well-correlated with the activity of the NADPH cytochrome c reductase (marker enzyme for the endoplasmic reticulum) and cytochrome c oxidase (marker enzyme for mitochondria), respectively. As detected by Fura-2 ratiometry, both inositol 1,4,5-trisphosphate (IP_3) and caffeine caused concentration-dependent Ca^{2+} releases from permeabilized cells and MC, but not from MT and SG. In an ATP-depleted condition, homogenates still took up a significant amount of Ca^{2+} but was not able to respond to IP_3 and caffeine. These results suggest that the endoplasmic reticulum is a major Ca^{2+} -storing organelle, which releases Ca^{2+} in response to IP_3 and caffeine in bovine adrenal chromaffin cells.

Keywords: Caffeine, Ca^{2+} store, Chromaffin cell, Inositol 1,4,5-trisphosphate, Organelle

It is widely accepted that Ca^{2+} plays a pivotal role in secretagogue-induced catecholamine secretion from adrenal chromaffin cells. For many years, it was thought that Ca^{2+} influx across the plasma membrane from the extracellular space was the only source of Ca^{2+} mobilization. However, using perfused cat adrenal glands, Nakazato et al. (1) found that muscarinic agonists, which stimulate IP_3 production in bovine adrenal medulla, evoked catecholamine secretion even in the absence of extracellular Ca^{2+} . In bovine adrenal chromaffin cells, IP_3 -mobilizing agonists (muscarinic agents, histamine or angiotensin II) were reported to increase the intracellular concentration of Ca^{2+} ($[\text{Ca}^{2+}]_i$) in the absence of extracellular Ca^{2+} , using fluorescent Ca^{2+} indicators Quin-2 or Fura-2 (2–5). On the other hand, caffeine, a powerful potentiator of Ca^{2+} -induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum in muscle, evoked Ca^{2+} mobilization and catecholamine secretion (4, 6, 7) in a Ca^{2+} -free condition, and both were blocked by the CICR channel inhibitor ryanodine in an use-dependent manner in bovine adrenal chromaffin cells (5, 8). Furthermore,

using digitonin-permeabilized cells of the bovine adrenal medulla, IP_3 was found to release Ca^{2+} from internal store sites (9), which was distinct from caffeine-sensitive stores (10, 11). The results of these studies indicate the existence of functional Ca^{2+} -storage sites, which are responsive to IP_3 or caffeine in adrenal chromaffin cells (12).

However, Ca^{2+} -storing organelles, which are sensitive to IP_3 or caffeine, are not always so clear in adrenal chromaffin cells. It was originally suggested that the IP_3 -sensitive store is the endoplasmic reticulum in rat pancreatic acinar cells (13). In accordance with this hypothesis, Kao (14) showed that the IP_3 -induced Ca^{2+} release was not affected by CCCP (carbonyl cyanide *m*-chlorophenylhydrazone), an inhibitor of mitochondrial Ca^{2+} uptake, in digitonin-permeabilized chromaffin cells of the bovine adrenal medulla. However, Yoo and Albanesi (15) reported that IP_3 evoked Ca^{2+} release not only from microsomal fractions but also from the fractions of secretory granules, which have been reported to accumulate Ca^{2+} in the physiological range of Ca^{2+} via an Na^+ -

Ca^{2+} exchange mechanism (16).

In the present study, we investigated the Ca^{2+} uptake and release properties of the fractions of microsomes, secretory granules, mitochondria and nuclei, in comparison with β -escin permeabilized cells and homogenates, to determine the organelles responsible for intracellular Ca^{2+} -storage, which are responsive to IP_3 or caffeine in adrenal chromaffin cells.

MATERIALS AND METHODS

Drugs

The following materials were purchased: adenosine 5'-triphosphate and β -escin from Sigma (St. Louis, MO, USA); collagenase type I from Worthington Biochemical (Freehold, NJ, USA); A23187 from Calbiochem (San Diego, CA, USA), glycoetherdiamine-*N,N,N',N'*-tetraacetic acid (EGTA), inositol 1,4,5-trisphosphate (IP_3) and piperazine-1,4-bis (2-ethanesulfonic acid) (PIPES) from Dojindo Laboratories (Kumamoto); caffeine monohydrate, sodium azide and thapsigargin from Wako Pure Chemical (Osaka). All reagents used were of commercial special grade.

Preparation of subcellular fractions, homogenates and β -escin permeabilized chromaffin cells

Subcellular fractions were prepared according to the method described by Azila and Hawthorne (17). Bovine adrenal medulla were homogenized in ice-cold 0.3 M sucrose solution (pH 7.0 adjusted by KOH) with a polytron (Kinematica, Littau, Switzerland) for 15 sec twice at volume 5. The homogenates were then filtered through nylon mesh (pore size, 250 μm) and the filtrate was centrifuged at $540 \times g$ for 10 min. The pellet was resuspended with sucrose solution and centrifuged again at low speed, and the resulting pellet resuspended with Cytosolution (described below) was the nuclear fraction. Supernatants, on the other hand, were centrifuged at $11,000 \times g$ for 20 min. The resulting supernatants were centrifuged at $42,000 \times g$ for 60 min, and the sediments were resuspended with Cytosolution (microsomal fraction). The sediments, after centrifugation at $11,000 \times g$, were largely a mixture of chromaffin granules and mitochondria, which were then layered on self-generating isopycnic gradients of percoll (Pharmacia, Uppsala, Sweden) at $11,000 \times g$ for 10 min. The self-generating gradients were previously prepared by centrifugation at $40,000 \times g$ for 30 min from 37.5% percoll dialyzed against 10 mM tris buffer (pH 7.0) overnight. The pellets at the bottom and interface were resuspended with Cytosolution, and the resulting specimens were the fractions of mitochondria and chromaffin granules, respectively. The homogenates were prepared in Cytosolution with a polytron homogenizer for 15 sec

twice at volume 5. The filtrates from the homogenates were used for the experiments. Adrenal chromaffin cells were isolated by collagenase digestion, and permeabilized according to the perviously described methods (8), except that the suspension medium was Cytosolution and β -escin (15 $\mu\text{g}/\text{ml}$) was used for cell permeabilization.

Measurement of Ca^{2+} uptake

Permeabilized cells and subcellular fractions in Cytosolution A containing 69 mM KCl, 4 mM MgCl_2 , 0.1 mM CaCl_2 and 20 mM PIPES (pH 7.0 adjusted with KOH) (ionic strength 0.16 M) were incubated at the protein concentration of 0.4–6 mg/ml, which showed linear relationships between the protein concentration and Ca^{2+} uptake, at 37°C with 5 mM ATP, desired concentrations of EGTA (pCa 8–4) and a trace amount of $^{45}\text{Ca}^{2+}$ (37 kBq/tube; Dupont, Boston, MA, USA). Incubation was terminated by filtering a 100- μl aliquot through a glass filter (pore size of 0.5 μm , GC50; Advantec, Tokyo) presoaked with wash solution, followed by two washes, each with a 2-ml aliquot of the same solution. The wash solution contained 130 mM KCl, 10 mM tris (hydroxymethyl) aminomethane and 5 mM MgCl_2 (pH 7.0 adjusted with HCl). $^{45}\text{Ca}^{2+}$ accumulation was measured by a liquid scintillation counter (LSC-400; Aloka, Tokyo). The concentrations of Ca^{2+} were calculated by solving multi-equilibrium equations using binding constants compiled by Martell and Smith (18). The presented data have been subtracted from the control values, which were obtained in the presence of 10 μM A23187, to eliminate passive adsorption.

Measurement of Ca^{2+} release

The free Ca^{2+} concentration was monitored ratiometrically using a Ca^{2+} indicator, Fura-2, in order to study the Ca^{2+} mobilizing activities of IP_3 and caffeine (19). Permeabilized cells and subcellular fractions were directly added into Cytosolution B, containing 69 mM KCl, 1.2 mM MgCl_2 and 20 mM PIPES (pH 7.0 adjusted with KOH), in a cuvette to a volume of 400 μl . Fluorescence was measured after the addition of 5 μM Fura-2 at 25°C using a dual-excitation fluorometer (CAF-110; Jasco, Tokyo). After Ca^{2+} was loaded into membrane preparations by the addition of both 1 mM ATP and 10 mM creatine phosphate plus 16 u/ml creatine phosphokinase (ATP regenerating system), IP_3 , caffeine and other drugs (up to 20 μl) were applied directly into the cuvette. The incubation mixture contained 5–10 mg of protein per 1 ml volume for each subcellular fraction. As mentioned elsewhere (20), caffeine potentiated the Fura-2 fluorescence regardless of the Ca^{2+} concentrations, although caffeine alone was not fluorescent. This potentiation by caffeine was more evident for F380 than for F340, with

the result that caffeine reduced the fluorescence ratio of Fura-2 (F340/F380) (see Results). For these reasons, Ca^{2+} levels are presented as the fluorescence ratio (F340/F380), not as concentration (mM), so that the vertical scales could be expressed in a more straightforward manner.

Assays of protein and organelle marker

The protein concentration was determined by the method of Lowry et al. (21). Cytochrome c oxidase (marker enzyme for mitochondria) was measured according to the method of Cooperstein and Lazarow (22). The activity of NADPH-cytochrome c reductase (marker enzyme for endoplasmic reticulum) was assayed as described by Williams Jr and Kamin (23). For the marker of chromaffin granules, the activities of dopamine- β -hydroxylase (DBH) were measured according the method of Nagatsu and Udenfriend (24).

Statistics

The data are presented as values of the arithmetic

mean $\pm$ S.E.M. Significance was determined by Student's *t*-test, and *P* values of less than 0.05 were considered to be statistically significant. EC_{50} values were calculated according to the Probit method.

RESULTS

Ca^{2+} uptake by organelle fractions, homogenates and permeabilized cells

In order to determine the relative significance of each organelle in the maintenance of cytosolic Ca^{2+} homeostasis, the uptake of Ca^{2+} by each organelle fraction was investigated in comparison with that of β -escin-permeabilized cells or homogenates using the $^{45}\text{Ca}^{2+}$ tracer method. Incubation of the individual membrane fractions including microsomes and mitochondria, homogenates or β -escin-permeabilized cells with $^{45}\text{Ca}^{2+}$ in the presence of 5 mM ATP led to a rapid accumulation of Ca^{2+} within 30 sec, and this reached a maximum at 10–20 min in a Ca^{2+} concentration-dependent manner

Fig. 1. $[\text{Ca}^{2+}]$ -dependent Ca^{2+} uptake by permeabilized cells, homogenates and various subcellular fractions from bovine adrenal chromaffin cells. β -Escin-permeabilized cells (a), homogenates (b) and the fractions of mitochondria (c), microsomes (d), secretory granules (e) and nuclei (f) from bovine adrenal medulla were incubated with various concentrations (10^{-8} – 10^{-4} M) of Ca^{2+} containing a trace amount of $^{45}\text{Ca}^{2+}$ at 37°C for 20 min in the presence (●) or absence (○) of 5 mM ATP. Symbols indicate the amount (nmol/mg protein) of net Ca^{2+} uptake, which was subtracted by the passive absorption obtained in the presence of $10 \mu\text{M}$ A23187. Data are values of the mean $\pm$ S.E.M. (bars) from 3 to 5 experiments. Magnifications of the low range of Ca^{2+} uptake below pCa 6.0 are also presented as insets in a–c.

Fig. 2. Correlation between Ca^{2+} uptake and marker enzyme activity of each organelle from bovine adrenal chromaffin cells under low and high Ca^{2+} conditions. Ca^{2+} uptake in the presence of 5 mM ATP by homogenates (HM) or the fractions of microsomes (MC), mitochondria (MT), secretory granules (SG), nuclei (NC) and a mixture of mitochondria and secretory granules before purification (MT+SG, f) are plotted as a function of the marker enzyme activity of several organelles at pCa 7.0 (a–c) and 5.8 (d–f). NADPH cytochrome c reductase (a, d), dopamine- β -hydroxylase (b, e) and cytochrome c oxidase (c, f) represent the marker activities for the endoplasmic reticulum, secretory granule and mitochondrion, respectively.

(pCa 8–5, Fig. 1). The nuclear fraction showed only slight uptake of Ca^{2+} (Fig. 1f); Uptake was largely inhibited by the presence of the Ca^{2+} -ATPase blocker thapsigargin (1 μM) (25). Even in the absence of ATP, which was completed by the addition of 16 u/ml hexokinase plus 20 mM glucose, permeabilized cells, homogenates and granular fractions were able to accumulate significant amounts of Ca^{2+} , although the amounts of Ca^{2+} uptake were minor (no more than 20% of the Ca^{2+} uptake in the presence of 5 mM ATP, Fig. 1: a, b and e). ATP-independent Ca^{2+} uptake was not observed at 0°C or Na^+ -dominant solution, suggesting that ATP-independent Ca^{2+} uptake by both preparations was evoked by secretory granules (16).

Correlation between Ca^{2+} uptake and marker enzyme activities for each organelle

Figure 2 shows the relationships between the amounts of Ca^{2+} accumulation and the marker enzyme activity

for each organelle at pCa 7.0 (a–c) and at pCa 5.8 (d–f). We used NADPH cytochrome c reductase as a marker enzyme for the endoplasmic reticulum, cytochrome c oxidase for mitochondria and dopamine- β -hydroxylase for secretory granules. As shown by Fig. 2 (a–c), only the activities of NADPH cytochrome c reductase were well-correlated with the Ca^{2+} uptake activities (correlation extinction: 0.94) at pCa 7.0, which is thought of as resting $[\text{Ca}^{2+}]_i$. At pCa 5.8, which is a possible $[\text{Ca}^{2+}]_i$ level to reach in response to maximal secretory stimuli in adrenal chromaffin cells (2, 26), only the activities of cytochrome c oxidase were well-correlated with the activities of Ca^{2+} uptake (correlation extinction: 0.99) (Fig. 2: d–f).

We confirmed that thapsigargin, an inhibitor for SERCA type Ca^{2+} -ATPase, selectively inhibits Ca^{2+} accumulation by the microsomal fraction by 60% (1 nM–1 μM) with an IC_{50} of about 5 nM, but has no effect on Ca^{2+} accumulation by mitochondrial or granular fractions. Thapsigargin (2.5 μM) selectively reduced Ca^{2+}

Fig. 3. The effect of IP₃ on Ca²⁺ mobilization from permeabilized cells or microsomal fractions of the bovine adrenal medulla. IP₃ (closed square: 100 nM–10 μM) was added to suspensions of permeabilized cells (a) or microsomes (b) at 25°C, after they were loaded with Ca²⁺ (40 nmol, a; 10 nmol, b). In some experiments, the effect of IP₃ was tested in the presence of heparin (Heparin, 200 μg/ml; horizontal line above the traces). Vertical bars and horizontal bars express the fluorescent ratio of Fura-2 (F340/F380) and the time scale (4 min), respectively.

accumulation by the permeabilized cell suspension or homogenate below pCa 6.5, but had no effect on those above 6.0. On the contrary, 1 mM NaN₃ (uncoupler) did not affect Ca²⁺ uptake by the microsomal or granular fractions, but almost abolished Ca²⁺ uptake by the mitochondrial fractions and permeabilized cell suspensions above pCa 6.0 (data not shown).

The effect of IP₃ on Ca²⁺ release from organelle fractions

The effects of IP₃ or caffeine on Ca²⁺ release from the fractions of microsomes, mitochondria or secretory granules, which showed Ca²⁺-storing ability, were investigated in comparison with β-escin-permeabilized cells or homogenates, by direct measurement of Ca²⁺ released using the fluorescent Ca²⁺ indicator Fura-2 (19).

The suspensions of permeabilized cells took up 40 nmol Ca²⁺ within a few minutes after the addition of ATP and the regenerating system, and reached a plateau (Ca²⁺ concentration of 400–700 nM, calculated by the procedure of Grynkiewicz et al., with 135 nM for the K_d value) (19). After that, the application of IP₃ caused a transient increase in the ambient concentration of Ca²⁺ (Fig. 3a). The amount of Ca²⁺ released was dependent on the concentration of IP₃ (20 nM–20 μM). Ca²⁺ release was observed from about 100 nM IP₃ and reached maximum at 5 μM with EC₅₀ values of 958 nM. The IP₃-activated Ca²⁺ channel inhibitor heparin (200 μg/ml) (27) almost abolished the response by 500 nM IP₃ and reduced that by

10 μM IP₃ to half. This inhibition was dependent on the concentration of heparin and the IC₅₀ value was 22.4 μg/ml against Ca²⁺ release by 500 nM IP₃. Qualitatively, the same results were obtained for the homogenate (data not shown). As shown by Fig. 3b, the basal level of Ca²⁺ reached a lower value (about 100 nM) in the microsomal suspension than in permeabilized cells, after they were loaded with 10 nmol Ca²⁺. IP₃ (20 nM–20 μM) was able to release Ca²⁺ from the microsomal fraction with a comparable time course and affinity to the releases from permeabilized cells or the homogenates (EC₅₀ values, 887 nM) (Fig. 3b). The effect of IP₃ was also inhibited by heparin, and the IC₅₀ values of this inhibitory effect resembled those of permeabilized cells and homogenates (18.7 μg/ml). However, IP₃, up to 20 μM, had no significant effect on the mitochondrial or secretory granule fractions after Ca²⁺ was accumulated by the application of ATP (Fig. 5: a and b).

The effect of caffeine on Ca²⁺ release from organelle fractions

As shown by Fig. 4a, the application of caffeine increased Ca²⁺ concentrations in the suspensions of permeabilized cells. The Ca²⁺ response was dependent on the concentration of caffeine and reached a maximum at 4–8 mM. The effects of caffeine were abolished by preincubating the cell suspensions with 20 mM caffeine plus 20 μM ryanodine at 37°C for 10 min, but not by preincuba-

Fig. 4. The effect of caffeine on Ca^{2+} mobilization from permeabilized cells or microsomal fractions of the bovine adrenal medulla. The effect of caffeine (closed square: 0.5–4 mM) was investigated in suspensions of permeabilized cells (a) and microsomal fractions (b).

tion with only one of the drugs, suggesting involvement of the ryanodine channel in this response (28) (data not shown). Caffeine rather reduced the fluorescence ratio of Fura-2, perhaps by a direct effect on the fluorescent properties of Fura-2 (20) (Fig. 4a). Similarly, the microsomal fraction released Ca^{2+} in response to caffeine with a similar affinity (0.5–8 mM) (Fig. 4b). However, the mitochondrial and secretory granule fractions were not able to respond to the release of Ca^{2+} upon the application of caffeine (Fig. 5: c and d).

As reported by Poulsen et al. (29), Ca^{2+} release by IP_3 (10 μM) or caffeine (4 mM) was largely inhibited by pretreatment with 2.5 μM thapsigargin before Ca^{2+} loading in permeabilized cells. We also obtained similar findings in the microsomal fraction. In accordance with Poulsen et al. (29), the magnitude of the inhibitory effects of thapsigargin was reduced by half when the drug was applied after Ca^{2+} loading. In the microsomal suspension, the application of 1 mM NaN_3 or replacement of KCl with NaCl in Cytosolution had no effect on the actions of both stimuli (data not shown).

The effects of IP_3 or caffeine under an ATP-depleted condition

The results presented above leave the possibility that secretory granules lose their sensitivities to IP_3 or caffeine through the purification procedure. Therefore, the effects of IP_3 and caffeine on the preparation of homogenates, loaded with Ca^{2+} beforehand in an ATP-depleted condition, were investigated, since only secretory granules

show an ATP-independent Ca^{2+} accumulation (16). As shown in Fig. 6, the homogenate still accumulated a considerable amount of Ca^{2+} even in the absence of ATP, which was completed by the addition of 16 u/ml hexokinase plus 20 mM glucose. Under this condition, the microsomal fractions did not show any Ca^{2+} uptake. Af-

Fig. 5. The effects of IP_3 and caffeine on Ca^{2+} mobilization from the secretory granule or mitochondrial fractions of the bovine adrenal medulla. IP_3 (10 μM) was added to suspensions of the secretory granules (a) and mitochondria (b). The effects of caffeine (4 mM) are shown by traces (c) (secretory granules) and (d) (mitochondria).

Fig. 6. The effects of IP_3 and caffeine on Ca^{2+} mobilization from homogenates in the absence of ATP. IP_3 (10 μM) and caffeine (4 mM) (Caf) were added into the homogenate preparations, which were previously loaded with 10 nmol Ca^{2+} in the presence (a, as a control) or absence (b) of 1 mM ATP. In b, residual ATP was metabolized by 16 u/ml hexokinase plus 20 mM glucose. Both suspensions contained an ATP regenerating system.

ter the Ca^{2+} level reached a plateau, however, application of 10 μM IP_3 or 4 mM caffeine did not increase the Ca^{2+} level, in spite of the fact that both of these drugs released Ca^{2+} from the homogenate in the presence of ATP (Fig. 6: a and b).

DISCUSSION

The Ca^{2+} -storing organelles in the adrenal medulla have been studied using the uncoupler CCCP or vanadate (nonselective Ca^{2+} -ATPase inhibitor), in digitonin-permeabilized cells (14). In organelles of adrenal chromaffin cells, however, the selectivity of these drugs has not been studied in detail. Therefore, in the present study, the relative role of each organelle in Ca^{2+} homeostasis was investigated by using the fractions of microsomes, mitochondria, secretory granules and nuclei in comparison with permeabilized cells or homogenates in the bovine adrenal medulla. The results suggested that the endoplasmic reticulum is the major Ca^{2+} -storing organelle in the resting state in bovine adrenal chromaffin cells. Purified

fractions of plasma membrane from bovine adrenal medulla have a high affinity uptake mechanism of Ca^{2+} (30). Although our microsomal fraction may contain a plasma membrane, it does not appear that most of the uptake of Ca^{2+} by the microsomal fraction was caused by contaminating plasma membrane, because there was no significant difference in the Ca^{2+} uptake activities between the homogenates and permeabilized cells. Furthermore, the amount of Ca^{2+} uptake by the plasma membrane (30) is equivalent to only 7% of that by the microsomal fraction in the present study.

The results of the present study also suggest that mitochondrion is the major Ca^{2+} -storing organelle at over pCa 6. This agrees well with the finding that CCCP-sensitive Ca^{2+} pools become active at above pCa 6 in permeabilized chromaffin cells (14). We also obtained the same results using 1 mM NaN_3 , another metabolic inhibitor (31) (data not shown). It still remains possible that mitochondria play a physiological role in chromaffin cells (32), as some investigators (26, 33) reported that $[\text{Ca}^{2+}]_i$ exceeds 1 μM especially in measurements at the single cell level, despite the fact that the real $[\text{Ca}^{2+}]_i$ can not be determined due to methodological problems (34). On the other hand, it was also reported that Ca^{2+} activates three dehydrogenases coupled to the electron transport system in mitochondria (31).

Since high amounts of Ca^{2+} within isolated chromaffin vesicles were reported, secretory granules are the best characterized organelle in chromaffin cells for Ca^{2+} accumulation, which is driven by a Na^+ - Ca^{2+} exchange but not by Ca^{2+} -ATPase, at least in part (16). In the present study, however, Ca^{2+} uptake by the granular fractions was only slight, and DBH, a marker enzyme for secretory granules, was not correlated with the activities of Ca^{2+} uptake in the presence of ATP for both low and high Ca^{2+} conditions. Furthermore, the capacity of ATP-independent Ca^{2+} uptake was less than 20% of that in the presence of ATP at pCa 7–5 for both permeabilized cells and homogenates. Therefore, it is unlikely that the secretory granules function as the major Ca^{2+} -storing organelle at the whole cell level. Otherwise Ca^{2+} within chromaffin granules may be important for stabilizing the structure of chromogranin A, catecholamine-packing protein (35), or Ca^{2+} accumulated by chromaffin granules may be released directly by exocytosis and may be useful for maintaining Ca^{2+} homeostasis in chromaffin cells during long-term stimulation (36).

In the present experiments, IP_3 and caffeine released Ca^{2+} only from microsomal fractions. As already mentioned, our microsomal fractions may contain a plasma membrane. In the plasma membranes of human T-lymphocytes (37) and rat mast cells (38), an IP_3 -activated Ca^{2+} current has been reported. However, it is unlikely

that both agonists stimulated Ca^{2+} release mainly from contaminating plasma membranes, since permeabilized cells released more Ca^{2+} than the homogenates by stimulation. Although Yoo and Albanesi (15) reported IP_3 -stimulated Ca^{2+} release from granular fractions, this was not observed in the present study. We also found that IP_3 or caffeine had no effects on permeabilized cells or homogenates previously loaded with Ca^{2+} under ATP-free conditions. The reason for this discrepancy is unknown. However, it seems unlikely that the secretory granules are the major Ca^{2+} store responsive to IP_3 or caffeine, because their capacity for Ca^{2+} uptake was minor as mentioned above. It was reported that an IP_3 -sensitive Ca^{2+} pool exists in the nuclei of the liver (39) and neurons (40). In the present study, the nuclear fraction of adrenal chromaffin cells showed a slight uptake of Ca^{2+} , which was sensitive to thapsigargin. However, there was no correlation between Ca^{2+} uptake activities and number of nuclei per ml, and neither IP_3 nor caffeine could release a significant amount of Ca^{2+} from the nuclear fractions (H. Teraoka et al., unpublished observations). These observations suggest that nuclei do not act as a major Ca^{2+} store in adrenal chromaffin cells, although it remains possible that the IP_3 -receptor channel is involved in intranuclear Ca^{2+} homeostasis (41). Poulsen et al. (29) showed that the receptors for IP_3 and ryanodine are co-distributed in subfractions of microsomes by immunoblotting using specific antibodies, which supports the results of the present study. On the other hand, Cheek et al. (10), and Robinson and Burgoyne (11) reported that IP_3 and caffeine released Ca^{2+} from discrete Ca^{2+} stores, while Stauderman et al. (5) suggested three types of Ca^{2+} stores: one exclusively sensitive to IP_3 , one sensitive to both IP_3 and caffeine, and another exclusively sensitive to caffeine. Therefore, multiple compartments within the endoplasmic reticulum may participate as a Ca^{2+} reservoir in response to IP_3 or caffeine (42), although further investigation is needed to clarify this possibility.

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