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EFFECTS OF RYANODINE ON CALCIUM SEQUESTRATION IN THE RAT LIVER

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Abstract—Ryanodine, a highly toxic alkaloid known to react specifically with the Ca^{2+} release channels in sarcoplasmic reticulum (SR), was employed to study Ca^{2+} sequestration in the liver. Ryanodine at a 200 μM concentration increased cytosolic free Ca^{2+} levels and phosphorylase *a* activity in isolated hepatocytes. These effects may involve microsomal Ca^{2+} sequestration, because ryanodine, in the presence of inhibitors of mitochondrial Ca^{2+} uptake, at concentrations of 1 nM, 1 μM, 50 μM and 100 μM decreased $^{45}Ca^{2+}$ retention in permeabilized hepatocytes. This inhibition of Ca^{2+} retention by ryanodine was not due to inhibition of the microsomal Ca^{2+}-ATPase. Dantrolene, a compound shown previously to inhibit ryanodine binding in the liver, also decreased $^{45}Ca^{2+}$ retention in permeabilized hepatocytes, and activated phosphorylase *a*. These results show that ryanodine administration alters calcium sequestration in liver. The possibility of the existence of a ryanodine-sensitive Ca^{2+}-release channel in liver is discussed.

Ryanodine, a toxic alkaloid, has been employed successfully in skeletal and cardiac muscle as an experimental tool to study the mechanism of Ca^{2+} release. Ryanodine was shown to cause irreversible contracture of skeletal muscle and to decrease the tension of cardiac muscle [1]. These results are due to the interaction of ryanodine with the heavy sarcoplasmic reticulum (SR), thereby modulating the Ca^{2+}-release activity of this membrane [2, 3]. The target molecule of ryanodine in muscle has been purified, reconstituted and found to form a Ca^{2+} channel [4].

Recently, we reported the presence of ryanodine binding sites also in the liver [5, 6]. In light of these data it seemed important to examine whether ryanodine binding in the liver also results in the modification of the distribution of Ca^{2+} as it does in the muscle. To answer this question we employed three different experimental approaches. We measured: (1) cystolic free Ca^{2+} levels with the Ca^{2+}-sensitive fluorescent indicator fura-2, (2) the activity of glycogen phosphorylase *a*, and (3) $^{45}Ca^{2+}$ retention in permeabilized hepatocytes.

MATERIALS AND METHODS

Animals. Fed, male Sprague–Dawley rats weighing 130–180 g, were used in all experiments.

Materials. Ethyleneglycol-bis (β-aminoethyl-ether)-*N*′,*N*′,*N*′,*N*′-tetraacetic acid (EGTA), 3(*N*-morpholino) propane sulfonic acid (MOPS), 2-(*N*-morpholino) ethanesulfonic acid (MES), trypan-blue, glycogen, caffeine, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), dithiothreitol (DTT), dantrolene, phenylephrine, ATP, phosphocreatine, creatine phosphokinase, ruthenium-red, and rotenone were obtained from the Sigma Chemical Co., St. Louis, MO. Collagenase (Type I) was purchased from the Worthington Biochemical Corp., Freehold, NJ. Glucagon was obtained from QUAD Pharmaceuticals, Inc., Indianapolis, IN. Albumin (bovine; Cohn fraction V) was purchased from the Biochemical Corp., Cleveland, OH. Glucose-1-phosphate was obtained from the Aldrich, Chemical Co., Milwaukee, WI; ryanodine from Calbiochem, La Jolla, CA; fura-2 from Molecular Probes, Inc., Eugene, OR; and $^{45}Ca^{2+}$ from ICN Radiochemicals, Irvine, CA. All others reagents were of the highest purity obtainable.

Preparation of isolated hepatocytes. Hepatocytes were isolated using the collagenase digestion technique [7] with some modifications. The liver was perfused *in situ* through the portal vein using Ca^{2+}-free Krebs–Ringer bicarbonate (KRB) containing 1 mM EGTA and 10 mM glucose. After 5 min of perfusion a regular KRB solution containing 2.54 mM $CaCl_2$, 10 mM glucose and 0.05% (w/v) collagenase was added to the perfusion apparatus and the perfusion continued until the liver surface looked soft. The liver was removed, placed in a petri dish containing regular KRB and collagenase, and minced. The liver suspension was filtered through cotton gauze. After filtration, cells were washed first with Ca^{2+}-free KRB containing EGTA and subsequently twice with regular KRB containing $CaCl_2$. The cells were resuspended in KRB containing $CaCl_2$, counted, and cell viability was assessed by trypan-blue exclusion.

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Isolation of microsomal fraction. Microsomes were isolated from livers of fasting male Sprague–Dawley rats. This method has been described in detail [8]. The liver was homogenized in 250 mM sucrose, 20 mM HEPES, 1 mM EGTA, and 1 mM DTT, pH 7.4. The homogenate was centrifuged at 1000 *g* for 10 min. The supernatant was centrifuged at 7700 *g* for 20 min and the resultant supernatant was recentrifuged at 100,000 *g* for 60 min. The pellet was resuspended in a medium containing 100 mM KCl, 20 mM HEPES, 1 mM DTT, pH 6.8.

$^{45}Ca^{2+}$ uptake and retention in permeabilized hepatocytes. Hepatocytes (1×10^6 cells/mL) were washed and resuspended in phosphate-buffered saline (PBS) and incubated on ice with saponin (0.02%) for 30 min. Permeabilized hepatocytes were then centrifuged, washed twice with PBS, and resuspended in a smaller volume of PBS. The permeabilized hepatocytes were then incubated at 37° for 15 min in a reaction mixture containing 19 mM $CaCl_2$ buffered with EGTA, $^{45}Ca^{2+}$ (1 μCi/mL), 2 mM ATP, and an ATP-regenerating system composed of phosphocreatine (5 mM) and creatine phosphokinase (5 units/mL), which also contained 10 μM ruthenium-red and 10 μM rotenone. An aliquot of the incubation mixture was filtered through 8.0 μm Millipore filters and washed, and the filters were subsequently counted in a scintillation counter.

Determination of phosphorylase a activity. Hepatocytes (3×10^6 cells/mL) were incubated for 30 min in KRB containing 2.54 mM $CaCl_2$ and 20 mM glucose. After 30 min, cell samples were taken and immediately frozen in liquid nitrogen. These samples were then homogenized in ice-cold buffer containing 0.5% glycogen, 100 mM NaF, 20 mM EDTA, 50 mM glycylglycine, and 20 mM MES, pH 6.1. Glycogen phosphorylase *a* activity was determined by the method of Stalmans and Hers [9], while the inorganic phosphate was assayed by the improved method of Lanzetta *et al.* [10].

Total protein. Protein determination for microsomes (Ca^{2+}-ATPase assay) or hepatocytes (phosphorylase *a* determination) was determined by the bicinchoninic acid (BCA, reagent obtained from the Pierce Chemical Co.) assay [11].

Determination of Ca^{2+}-ATPase activity. The activity was determined as previously described [8]. The incubation medium contained 100 mM KCl, 20 mM HEPES/KOH, pH 6.8, 1 mM ouabain, 2 mM EGTA, 1 mM NaN_3, 1 μM ionophore A23187, 5 mM $MgCl_2$, 2.6 mM $CaCl_2$ (to produce a free Ca^{2+} and Mg^{2+} concentration of 0.2 and 2.5 mM, respectively) and microsomal protein. The reaction was started by adding 5 mM ATP. After incubation at 37° for 10 min, the reaction was stopped by adding ice-cold 16% (w/v) trichloroacetic acid. The protein-free supernatant obtained after centrifugation at 5000 *g* for 5 min was assayed for inorganic phosphorus using the colorimetric method described by Lanzetta *et al.* [10].

Cytosolic free Ca^{2+} concentration measurement using fura-2 AM. Hepatocytes (0.5×10^6 cells/mL) loaded with acetoxymethylester of fura-2 (5 μM) (fura-2 AM) in dimethyl sulfoxide (DMSO) and 0.02% pluronic acid F-127 [12] were incubated in a buffer containing 142 mM NaCl, 3 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 1 mM KH_2PO_4, 20 mM HEPES, 0.2% albumin, and 10 mM glucose for 1 hr on a rocker at room temperature while gassed with O_2/CO_2 (19:1). After incubation, the cells were washed and centrifuged twice at room temperature at 500 *g* for 1 min and resuspended in a smaller volume of the above buffer.

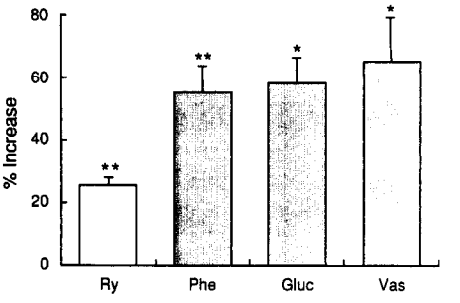

Fig. 1. Effects of 200 μM ryanodine (Ry), 10^{-5} M phenylephrine (Phe), 10^{-8} M glucagon (Glu) and 10^{-9} M vasopressin (Vas) on cytosolic free calcium concentration. Cytosolic free calcium was measured by the fura-2 method as described in Materials and Methods. The data are means ± SEM of three to eight experiments using different preparations of hepatocytes. The results are expressed as percent increase from the basal value, which corresponded to 463 ± 71 nM Ca^{2+} in ten experiments. Statistically significant increase from basal values: (*) $P < 0.05$, (**) $P < 0.01$.

The fluorescence of fura-2 loaded cells was measured at 37° by using excitation and emission wavelengths of 340 and 505 nm, respectively. $[Ca^{2+}]_i$ was calculated by using the equation given in Ref. 12 and a K_d of 242 for the dissociation constant. Values for F_{max} and F_{min} of cells loaded with fura-2 were obtained using 0.2% Triton and 0.3 M EGTA and a saturated Tris solution.

The data were analyzed by a paired *t*-test using a computer program (Primer Biostatistics: The Program).

RESULTS

The effect of ryanodine on cytosolic free Ca^{2+} levels, as indicated by measurements with fura-2, is shown in Fig. 1. In these, as well as in the next series of experiments where glycogen phosphorylase *a* activity was measured, high concentrations of ryanodine were employed. The reason for choosing a high concentration of ryanodine in these experiments, carried out with intact cells, is the high polarity of the ryanodine molecule. Thus, it seems that ryanodine is unlikely to easily penetrate the liver cell membrane. Because we cannot determine how much of the ryanodine actually gets into the cell and reaches its site of action, the purpose in these experiments was limited to establishing whether or not ryanodine has an effect. As shown in Fig. 1, ryanodine caused a rather small, but statistically significant increase in cytosolic free Ca^{2+} levels. The increase observed with ryanodine, however, was less than the increase obtained with the hormones,

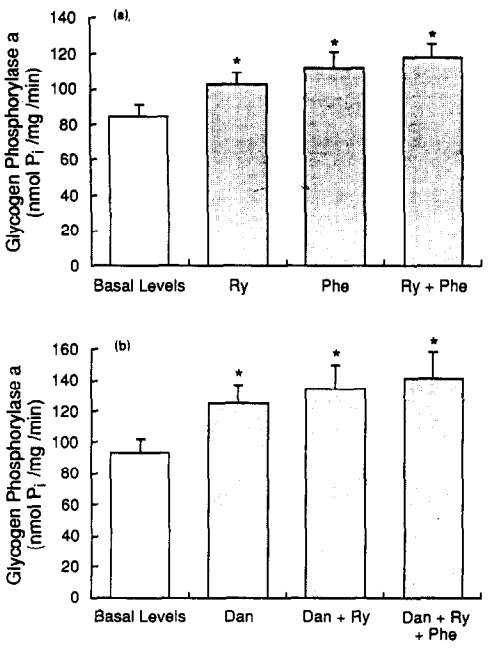

Fig. 2. Effect of 10^{-5} M phenylephrine (Phe), 200 μM ryanodine (Ry) and 100 μM dantrolene (Dan) on glycogen phosphorylase *a* activity. Glycogen phosphorylase *a* activity was determined as described in Materials and Methods. The data are means ± SEM of at least six experiments using different preparations of hepatocytes. Statistically significant increase from basal values: (*) $P < 0.05$.

glucagon and vasopressin, and the α-adrenergic agent, phenylephrine. The increase in $[Ca^{2+}]_i$ after ryanodine administration proceeded at a somewhat slower rate, taking 1 min, in contrast to the immediate increase observed with the hormones taking only seconds (results not shown).

An accepted indicator of $[Ca^{2+}]_i$ is the activation state of glycogen phosphorylase *a* [13–16]. Thus, to establish the effect of ryanodine on $[Ca^{2+}]_i$ by an additional method, its effect on the activation of this enzyme was measured. As shown in Fig. 2a, ryanodine activated phosphorylase *a*, though less effectively than phenylephrine did. The effects of phenylephrine and ryanodine seem to be additive.

Dantrolene, a muscle relaxant drug, was shown to suppress Ca^{2+} release by the SR [17], though it does not seem to affect Ca^{2+}-induced Ca^{2+} release [18], and was also shown previously to inhibit ryanodine binding in the liver [5, 6]. It was of interest, therefore, to check its influence by itself and in combination with ryanodine on Ca^{2+} sequestration. Its effect could be measured only on glycogen phosphorylase *a* activity because its strong yellow coloring interfered in our system with the determination of $[Ca^{2+}]_i$ by means of fura-2 measurement.

As shown in Fig. 2b, dantrolene by itself activated glycogen phosphorylase *a* even more effectively than ryanodine did. The effect of dantolene was due to the drug and not to the vehicle solvent (DMSO) which, by itself, had no effect on the activity of the enzyme (results not shown).

In the above experiments the permeability of ryanodine across the liver cell plasma membrane may have posed a limiting factor to its effectiveness. To bypass this difficulty, in subsequent experiments saponin-permeabilized hepatocytes were employed. Digitonin- or saponin-permeabilized cells have been proven to be useful tools in studies on Ca^{2+} sequestration [19–22]. The saponin-permeabilized hepatocytes took up $^{45}Ca^{2+}$ in an ATP-dependent manner in the presence of rotenone and of ruthenium-red, an inhibitor of mitochrondrial-Ca^{2+} uptake. In contrast to the skeletal muscle, ruthenium-red does not inhibit ryanodine binding in the liver [5, 6]. In separate experiments, addition of 20 mM oxalate stimulated $^{45}Ca^{2+}$ uptake 2-fold, which confirmed that the uptake of $^{45}Ca^{2+}$ was into the endoplasmic reticulum (results not shown). However, because we were interested in release rather than uptake, oxalate was omitted from the incubation medium in the experiments with ryanodine and dantrolene. As shown, these two drugs inhibited $^{45}Ca^{2+}$ accumulation by the hepatocytes (Table 1). The effect of ryanodine was significant at a concentration of 1×10^{-9} M and above. However, the increase in the effect was not proportional with the increases in concentration. Dantrolene, at much higher concentrations, also reduced $^{45}Ca^{2+}$ accumulation.

The inhibition of $^{45}Ca^{2+}$ accumulation could be due either to inhibition of the uptake or to stimulation of release. To distinguish between these two possibilities, the effect of ryanodine on the activity of microsomal Ca^{2+}-ATPase was measured. Ryanodine at concentrations which reduced $^{45}Ca^{2+}$ accumulation did not affect the activity of the microsomal Ca^{2+}-ATPase (Table 2).

DISCUSSION

The data presented are compatible with the suggestion that ryanodine has a significant effect on Ca^{2+} sequestration in the liver. The conclusion is based on three observations: (1) ryanodine caused an increase in $[Ca^{2+}]_i$ as measured by the Ca^{2+} indicator, fura-2; (2) it induced an increase in activity of glycogen phosphorylase *a*; and (3) ryanodine also inhibited $^{45}Ca^{2+}$ uptake in permeabilized hepatocytes.

The pattern of increase in $[Ca^{2+}]_i$ observed after ryanodine administration, as measured by fura-2, resembled more closely the pattern of gradual increase observed following the administration of halothane to the liver [23] than that of the hormones, which is immediate. This observation may be relevant because halothane in skeletal muscle was shown to modify the activity of the Ca^{2+}-induced Ca^{2+} release channel, which is ryanodine sensitive [2, 3, 24–26]. The polarity of the ryanodine molecule may also be a contributing factor; it may take minutes for it to get into the cell, whereas it only takes seconds for the hormones to activate the Ca^{2+} release process.

Because the increase in $[Ca^{2+}]_i$ following ryanodine administration as registered by an increase in fura-2 fluorescence is rather small, it was important to verify the observation by using a different method

Table 1. Effects of ryanodine and dantrolene on $^{45}Ca^{2+}$ uptake in permeabilized hepatocytes

Concentration	Calcium uptake (nmol $Ca^{2+}/10^6$ cells)			% Inhibition
	Control	Ryanodine	Dantrolene	
0.01 nM	2.23 ± 0.50	2.17 ± 0.28 (NS)*		3
0.1 nM	1.96 ± 0.34	2.03 ± 0.49		0
1.0 nM	2.29 ± 0.48	2.01 ± 0.47†		12
1.0 μM	2.15 ± 0.39	1.59 ± 0.29†		26
50.0 μM	1.75 ± 0.68	1.33 ± 0.59†		24
20 μM	2.05 ± 0.40		1.71 ± 0.21 (NS)	17
50 μM	2.05 ± 0.40		1.66 ± 0.32†	19
100 μM	2.26 ± 0.41		1.85 ± 0.40†	18
200 μM	2.62 ± 0.44		1.59 ± 0.22†	40

Ca^{2+} uptake was determined as described in Materials and Methods. The data are means ± SEM of at least three separate experiments using different preparations of permeabilized hepatocytes.

* NS = not significant.

† Significantly different from control at $P < 0.05$.

Table 2. Effect of ryanodine (Ry) on Ca^{2+}-ATPase in hepatic microsomal fraction

Treatment	Ca^{2+}-ATPase (nmol P_i/mg/min)	Significance
Control	130.3 ± 4.81	
+ Ry (1 nm)	121.2 ± 10.97	P = 0.292 (NS)*
+ Ry (100 nM)	120.0 ± 15.89	P = 0.461 (NS)
+ Ry (1 mM)	116.0 ± 20.32	P = 0.453 (NS)

Ca^{2+}-ATPase was determined as described in Materials and Methods. The data are means ± SEM of three experiments.

* NS = not significant.

of measurement. We chose to measure the activation of glycogen phosphorylase *a* as an additional indicator of $[Ca^{2+}]_i$. The activation of the enzyme by ryanodine means an increase in $[Ca^{2+}]_i$, and thus reinforced the results obtained with the fura-2 measurements.

In the above experiments high concentrations of ryanodine were used. This was necessitated by the fact that we were interested in a fast response and there was no way of knowing how much of the drug actually entered the cells. Thus, the possibility that ryanodine actually acted on the plasma membrane and stimulated Ca^{2+} influx cannot be excluded. However, the increase in $[Ca^{2+}]_i$ following ryanodine administration to hepatocytes is likely to result, at least in part, from the release of stored intracellular Ca^{2+}. This conclusion is based on the inhibitory effect of ryanodine on $^{45}Ca^{2+}$ retention in permeabilized hepatocytes loaded with $^{45}Ca^{2+}$ in the presence of ATP and inhibitors of mitochondrial Ca^{2+} uptake (Table 1). Because ryanodine did not inhibit Ca^{2+}-ATPase (Table 2), the decrease in $^{45}Ca^{2+}$ content was not caused by an inhibition of the uptake. Ryanodine must, therefore, stimulate the release of $^{45}Ca^{2+}$, resulting in less accumulation.

The effect of ryanodine on $^{45}Ca^{2+}$ uptake in permeabilized cells is significant at nanomolar concentrations. The apparent K_d for ryanodine in isolated microsomal vesicles is 10 ± 2 nM [6]. Thus, ryanodine is effective at concentrations within the range of the K_d. Under the experimental conditions employed, ryanodine is likely to release $^{45}Ca^{2+}$ from the endoplasmic reticulum because mitochondrial Ca^{2+} uptake was inhibited. However, it is evident from the data that the ryanodine effect in this preparation does not show the expected concentration-dependency. Thus, whether this release represents an action of the drug at the specific release site is unresolved. The endoplasmic reticulum is a major calcium storage site in the liver [27]; however, the possibility that ryanodine releases $^{45}Ca^{2+}$ from additional compartments cannot be excluded.

It is relevant to the present studies that in previous experiments Mine *et al.* [28] reported that 500 μM dantrolene inhibited phenylephrine-evoked Ca^{2+} efflux and also the increase in cytosolic free Ca^{2+} level. These effects of dantrolene indicated a possible dantrolene-induced inhibition of a Ca^{2+} channel. It seems possible that, depending on the experimental conditions, binding of dantrolene may also result in the opening of the same channel.

The data presented demonstrate, unequivocally, that ryanodine alters Ca^{2+} sequestration in the liver, probably by causing the release of accumulated Ca^{2+} from a storage pool. Thus, even though the characteristics of ryanodine binding in the liver are very different from those of the skeletal muscle and the binding site may be a different molecule [6], the implications of the binding seem to be similar: alteration of Ca^{2+} sequestration. The identification of the ryanodine-binding protein and the question of whether it has a physiological function in the liver remains to be elucidated.

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REFERENCES

1. Jenden DJ and Fairhurst AS, The pharmacology of ryanodine. *Pharmacol Rev* **21**: 1–25, 1969.
2. Lai FA and Meissner G, The muscle ryanodine receptor and its intrinsic Ca^{2+} channel activity. *J Bioenerg Biomembr* **21**: 227–245, 1989.
3. Fleischer S and Inui M, Biochemistry and biophysics of excitation–contraction coupling. *Annu Rev Biophys Biophys Chem* **18**: 333–364, 1989.
4. Lai FA, Erickson HP, Rousseau E, Liu Q-Y and Meissner G, Purification and reconstitution of the calcium release channel from skeletal muscle. *Nature* **331**: 315–319, 1988.
5. Shoshan-Barmatz V, Zhang GH, Garretson L and Kraus-Friedmann N, Distinct ryanodine- and inositol 1,4,5-trisphosphate-binding sites in hepatic microsomes. *Biochem J* **268**: 699–705, 1990.
6. Shoshan-Barmatz V, Pressley TA, Higham S and Kraus-Friedmann N, Characterization of high-affinity ryanodine-binding sites of rat liver endoplasmic reticulum. Differences between liver and skeletal muscle. *Biochem J* **276**: 41–46, 1991.
7. Berry MN and Friend DS, High-yield preparation of isolated rat liver parenchymal cells. A biochemical and fine structural study. *J Cell Biol* **43**: 506–520, 1969.
8. Zhang GH and Kraus-Friedmann N, The effects of Mg^{2+} on rat liver microsomal Ca^{2+} sequestration. *Cell Calcium* **11**: 397–403, 1990.
9. Stalmans W and Hers H-G, The stimulation of liver phosphorylase *b* by AMP, fluoride and sulfate. A technical note on the specific determination of the *a* and *b* forms of liver glycogen phosphorylase. *Eur J Biochem* **54**: 341–350, 1975.
10. Lanzetta PA, Alvarez LJ, Reinach PS and Candia OA, An improved assay for nanomole amounts of inorganic phosphate. *Anal Biochem* **100**: 95–97, 1979.
11. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**: 76–85, 1985.
12. Grynkiewicz G, Poenie M and Tsien RY, A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* **260**: 3440–3450, 1985.
13. Blackmore PF, Hughes BP, Shuman EA and Exton JH, α-Adrenergic activation of phosphorylase in liver cells involves mobilization of intracellular calcium without influx of extracellular calcium. *J Biol Chem* **257**: 190–197, 1982.
14. Suresh KJ, Coll KE, Cooper RH, Marks JS and Williamson JR, Mechanisms underlaying calcium homeostasis in isolated hepatocytes. *J Biol Chem* **258**: 731–741, 1983.
15. Bellomo G, Nicotera P and Orrenius S, Alterations in intracellular calcium compartmentation following inhibition of calcium efflux from isolated hepatocytes. *Eur J Biochem* **144**: 19–23, 1984.
16. Starke PE, Hoek JB and Farber JL, Calcium-dependent and calcium-independent mechanisms of irreversible cell injury in cultured hepatocytes. *J Biol Chem* **261**: 3006–3012, 1986.
17. Van Winkle WB, Calcium release from skeletal muscle sarcoplasmic reticulum: Site of action of dantrolene sodium? *Science* **193**: 1130–1131, 1976.
18. Nelson TE, Dantrolene does not block calcium pulse-induced calcium release from a putative calcium channel in sarcoplasmic reticulum from malignant hyperthemia and normal pig muscle. *FEBS Lett* **167**: 123–126, 1984.
19. Streb H, Irvine RF, Berridge MJ and Schulz I, Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol 1,4,5-trisphosphate. *Nature* **306**: 67–69, 1983.
20. Burgess GM, McKinney JS, Fabiato A, Leslie BA and Putney JW Jr, Calcium pools in saponin-permeabilized guinea pig hepatocytes. *J Biol Chem* **258**: 15336–15345, 1983.
21. Joseph SK, Thomas AP, Williams RJ, Irvine RF and Williamson JR, *myo*-Inositol 1,4,5-trisphosphate. A second messenger for the hormonal mobilization of intracellular Ca^{2+} in liver. *J Biol Chem* **259**:3077–3081, 1984.
22. Thomas AP, Enhancement of the inositol 1,4,5-trisphosphate-releasable Ca^{2+} pool by GTP in permeabilized hepatocytes. *J Biol Chem* **263**: 2704–2711, 1984.
23. Iaizzo PA, Olsen RA, Seewald MJ, Powis G, Stier A and Van Dyke RA, Transient increase of intracellular Ca^{2+} induced by volatile anesthetics in rat hepatocytes. *Cell Calcium* **11**: 515–524, 1990.
24. Nelson TE and Sweo T, Ca^{2+} uptake and Ca^{2+} release by skeletal muscle sarcoplasmic reticulum: Differing sensitivity to inhalational anesthetics. *Anesthesiology* **69**: 571–577, 1988.
25. Nelson TE, Ryanodine: Antithetical calcium channel effects in skeletal muscle sarcoplasmic reticulum. *J Pharmacol Exp Ther* **242**: 56–61, 1987.
26. Bianchi CP and Narayan S, Bimodal operation of the ryanodine-sensitive transducer calcium channel. *Toxicon* **28**: 1173–1181, 1990.
27. Somlyo AP, Bond M and Somlyo AV, Calcium content of mitochondria and endoplasmic reticulum in liver frozen rapidly *in vivo*. *Nature* **314**: 622–625, 1985.
28. Mine T, Kojima I, Kimura S and Ogata E, Assessment of the role of Ca^{2+} mobilization from intracellular pool(s), using dantrolene, in the glycogenolytic action of α-adrenergic stimulation in perfused rat liver. *Biochim Biophys Acta* **927**: 229–234, 1987.