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Ionic requirements for inositol trisphosphate- and thapsigargin-induced Ca^{2+} release from rat liver endoplasmic reticulum

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ABSTRACT: The Ca^{2+}-transport and permeability properties of the endoplasmic reticulum (ER) from rat liver were studied by manipulating the ionic composition of the external medium. The ATP-dependent Ca^{2+} loading into the ER vesicles was slow in the absence of K^+ and the amount of Ca^{2+} absorbed was also limited. However, the presence of gluconate in the medium greatly enhanced the Ca^{2+}-loading capacity of the vesicles. Ca^{2+} release from the vesicles was triggered by inositol (1,4,5) trisphosphate in the presence of GTP or by thapsigargin. While the Ins(1,4,5)P_3-induced Ca^{2+} release was dependent on the presence of K^+ in the external medium, thapsigargin released Ca^{2+} from the vesicles irrespective of the ionic composition of the external medium. These findings suggest that a specific ion channel is not involved in the release of Ca^{2+} from ER vesicles by thapsigargin.

Key Words: Ca^{2+} transport; Endoplasmic reticulum; Microsomes; Thapsigargin; Inositol (1,4,5) trisphosphate.

In rat liver endoplasmic reticulum, the Ins (1,4,5) P_3-stimulated Ca^{2+} release is greatly enhanced by low concentrations of GTP, in the presence or absence of polyethylene glycol (1,2). Dawson . (3) have shown that GTP mediates a slow but extensive Ca^{2+} mobilization. However, these effects are not observed in the absence of potassium and chloride as counterions.

In recent times, the sesquiterpene lactone, thapsigargin, has become a useful tool in characterizing the Ca^{2+} release process from the ER (4-6). This compound selectively produces a hormone-independent Ca^{2+}-release from the ER by bypassing the membrane hydrolysis of phosphoinositides and acting directly on the intracellular store (5).

The present work was designed to see whether the presence of KCl in the external medium is also necessary for the release of Ca^{2+} from ER vesicles by thapsigargin.

ATP (vanadate-free) and dithiothreitol were from Boehringer-Mannheim, UK Ltd. Thapsigargin, choline chloride, magnesium gluconate, potassium gluconate, phosphocreatine, creatine kinase, polyethylene glycol and Hepes were purchased from Sigma Chemical Co., Poole, Dorset, UK. Ins(1,4,5)P_3 was a gift from Dr. R. F. Irvine, AFRC Institute of Animal Physiology, Babraham, Cambridge, UK. All other reagents were of analytical grade.

Rat liver microsomes (36,000g fraction) were prepared from fed male albino rats as described by Dawson and Fulton (7). The protein concentrations of the microsomal fractions were determined by the procedure of Lowry . (8). Ca^{2+} uptake and release were monitored using the fluorescent Ca^{2+} indicator Fluo 3 as described by Comerford and Dawson (9), with slight modifications. Ca^{2+} uptake was initiated by the addition of microsomes (1.5 mg/ml final concentration) to the reaction vessel containing 150 mM sucrose, 50 mM KCl, 10 mM

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Hepes/KOH (pH 7.0), 5% (w/v) polyethylene glycol (PEG), 1 mM dithiothreitol, 5 mM ATP, 2 mM $MgCl_2$, 10 mM phosphocreatine, 10 µg/ml creatine kinase and Fluo 3 (0.67 µM final concentration). This basic medium was varied, to suit various experimental conditions, by the replacement of K^+ with Na^+ or choline, and Cl^- with gluconate, as described in the legends to the figures.

GTP-dependent Ca^{2+} release was initiated by the addition of GTP (50 µM final concentration). Ins(1,4,5)P_3-dependent Ca^{2+} release was also initiated by the addition of Ins(1,4,5)P_3 to a final concentration of 2 µM. Fluorescence intensity was measured at 30^{o}C using a Shimadzu RF 5000 spectrofluorimeter with an excitation wavelength of 505 nm and emission wavelength at 530 nm.

In the control experiment (Fig. 1a) Ins(1,4,5)P_3 was found to release Ca^{2+} from microsomal vesicles after ATP-dependent Ca^{2+} loading in a medium containing KCl. Subsequent experiments (Figs. 1b, 1c and 2a, b, c) showed that the uptake of Ca^{2+} into the vesicles varied according to the ionic composition of the surrounding medium. Substitution of K^+ with Na^+ did not affect the capacity of the vesicles to load Ca^{2+}. However, substitution of K^+ with choline or Cl^- with gluconate drastically reduced the rate of Ca^{2+} loading into the vesicles, as well as the total amount of Ca^{2+} loaded. These substitutions also enhanced the GTP-dependent Ca^{2+}-mobilization and the Ins(1,4,5)-induced Ca^{2+} release from the ER vesicles (Fig. 2).

In the experiment shown in Fig. 3, thapsigargin was used to release Ca^{2+} from the microsomal vesicles after an initial Ca^{2+} loading in various ionic media. Thapsigargin released Ca^{2+} from the vesicles, irrespective of the ionic composition of the external medium. The rate and extent of Ca^{2+} loading was, however, influenced by the external medium.

It is now generally accepted that the turnover (synthesis and hydrolysis) of polyphospho-inositides, provoked by a wide variety of extracellular messengers such as neurotransmitters, hormones, growth factors and many other biologically active substances, is the signal for the transmembrane control of some cellular processes (10 - 13). The hydrolysis of phosphatidylinositol 4,5-bisphosphate [PtdIns (4,5)P_3] produces two intracellular messengers: 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]. The former activates protein kinase C while the latter releases Ca^{2+} from intracellular stores by activating a Ca^{2+} channel (11).

The intracellular Ca^{2+} stores have been shown to be heterogeneous in character and various investigators have used calcium pump inhibitors such as thapsigargin and 2,5-di-(tert-butyl)-1,4-benzohydroquinone (DBHQ) to characterize the different Ca^{2+} stores (14,15).

The results obtained inthe present study show that the ionic composition of the external medium influences the rate and extent of Ca^{2+} uptake by microsomal vesicles. This is in agreement with the concept that Ca^{2+} is transported into the vesicles through voltage-sensitive Ca^{2+}-channels which are controlled by the electrical potentials across the membranes (16).

The results presented in Figs. 1 and 2 show [redacted] the microsomal vesicles, the GTP-dependent Ca^{2+} mobilization by the vesicles as well as the Ins(1,4,5)P_3-induced Ca^{2+} release by the vesicles are all functions of the ionic composition of the external medium. This is very well illustrated by the slow and very limited Ca^{2+} uptake and the lack of GTP and Ins(1,4,5)P_3 responses shown in Fig. 1c. Fig. 2 shows that all these responses are enhanced in the presence of Na^+ and/or Cl^- ions.

The fact that thapsigargin releases Ca^{2+} from the microsomal vesicles, irrespective of the ionic composition of the external medium, suggests that the release pathway is not through the voltage-sensitive Ca^{2+} channel which is regulated by the electrochemical potential gradient across the membranes. While the characteristics of the active Ca^{2+} uptake by the endoplasmic reticulum have been well studied, the mechanism by which Ca^{2+} is released in response to the depolarization of the membrane is still not well understood. Several mechanisms have been proposed for the Ca^{2+} release process, the most interesting among them being the depolarization-induced Ca^{2+} release and the Ca^{2+}-induced Ca^{2+} release. The first mechanism is supported by the results of the present study in which transient potentials were induced across the membranes by manipulating the electrolyte composition of the reaction medium.

[redacted] thapsigargin and Ins(1,4,5)P_3 on the Ca^{2+} release process from the endoplasmic reticulum and other intracellular Ca^{2+} stores would throw more light on the mechanism(s) of Ca^{2+} uptake and release by various intracellular Ca^{2+} transport systems.

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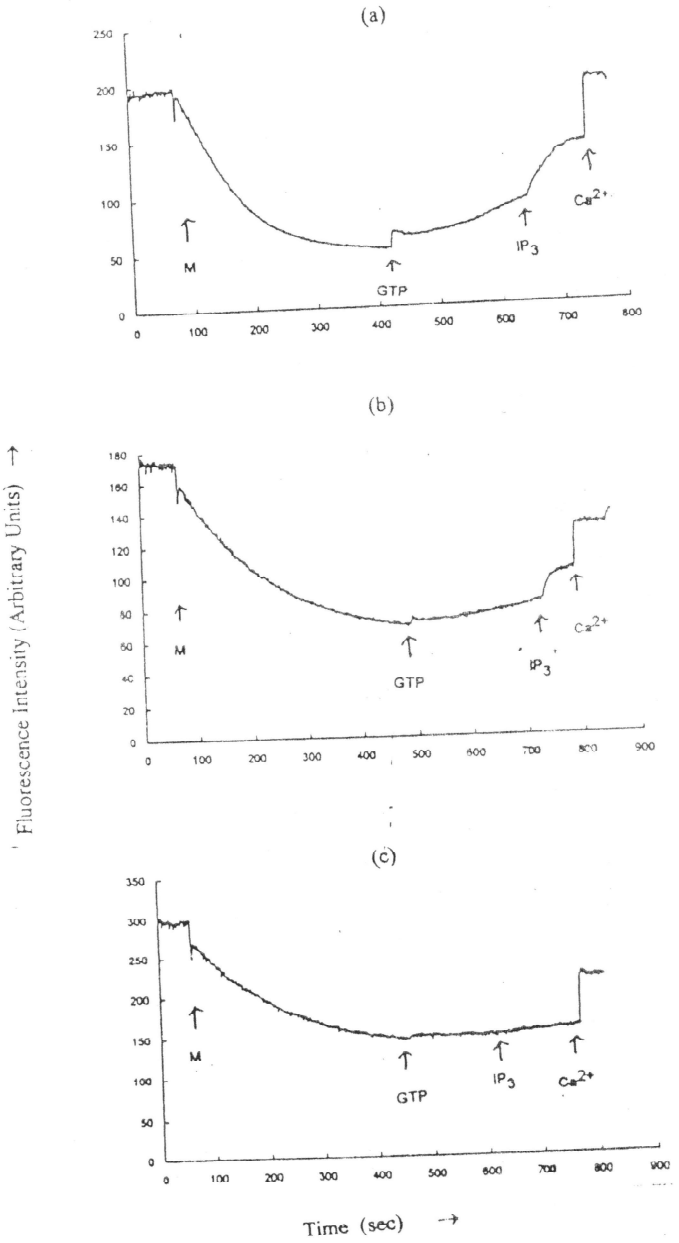

Fig. 1: Effects of potassium and chloride ions on Ca^{2+} uptake and release in rat liver microsomal vesicles.

Microsomes (1.5 mg/ml) were incubated in (a) the basic reaction medium described in the "Materials and Methods" section, (b) a solution in which KCl and $MgCl_2$ in the basic medium were replaced with K-gluconate (50 mM) and Mg-gluconate (2 mM) respectively, (c) a solution in which $MgCl_2$ in the basic medium was replaced with Mg-gluconate (2 mM) and KCl was omitted without replacement. Arrows

indicate where the following were added: M, microsomes; G, GTP (50μM); IP_3, $Ins(1,4,5)P_3$ (2μM); Ca^{2+}, calcium ions (10 nmoles). Traces were obtained from data generated by Shimadzu RF 5000 software which were subsequently fed into Microsoft Excel software.

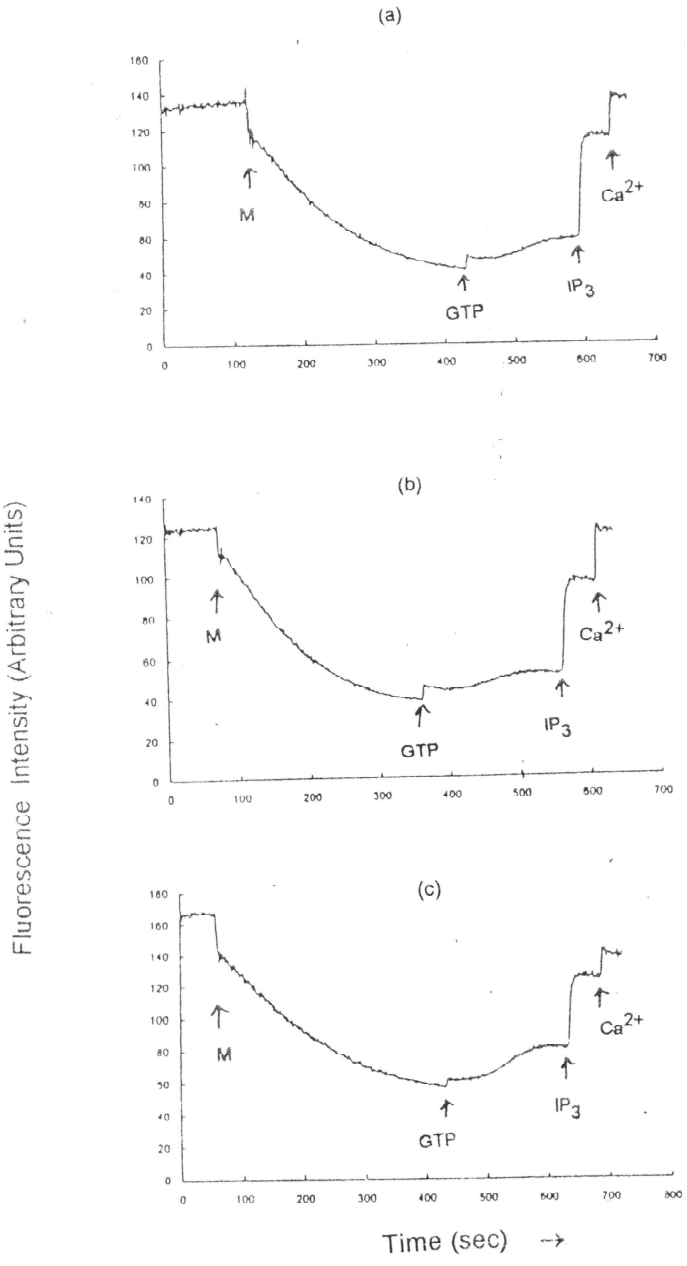

Fig. 2: Effect of sodium ions on Ca^{2+} uptake and release in rat liver microsomal vesicles.

Microsomes (1.5 mg/ml) were incubated in (a) a solution in which KCl in the basic medium described in the "Materials and Methods" section was replaced with NaCl (50 mM), (b) a solution in which KCl in the basic medium was replaced with choline chloride (50 mM), (c) a solution in which KCl in the basic medium was replaced with Na-gluconate (50 mM) and $MgCl_2$ was replaced with Mg-gluconate (2 mM) and KCl was omitted without replacement. Arrows indicate where the following were added: M, microsomes; G, GTP (50μM); IP_3, Ins(1,4,5)P_3 (2μM); Ca^{2+}, calcium ions (10 nmoles). Traces were obtained from data generated by Shimadzu RF 5000 software which were subsequently fed into Microsoft Excel software.

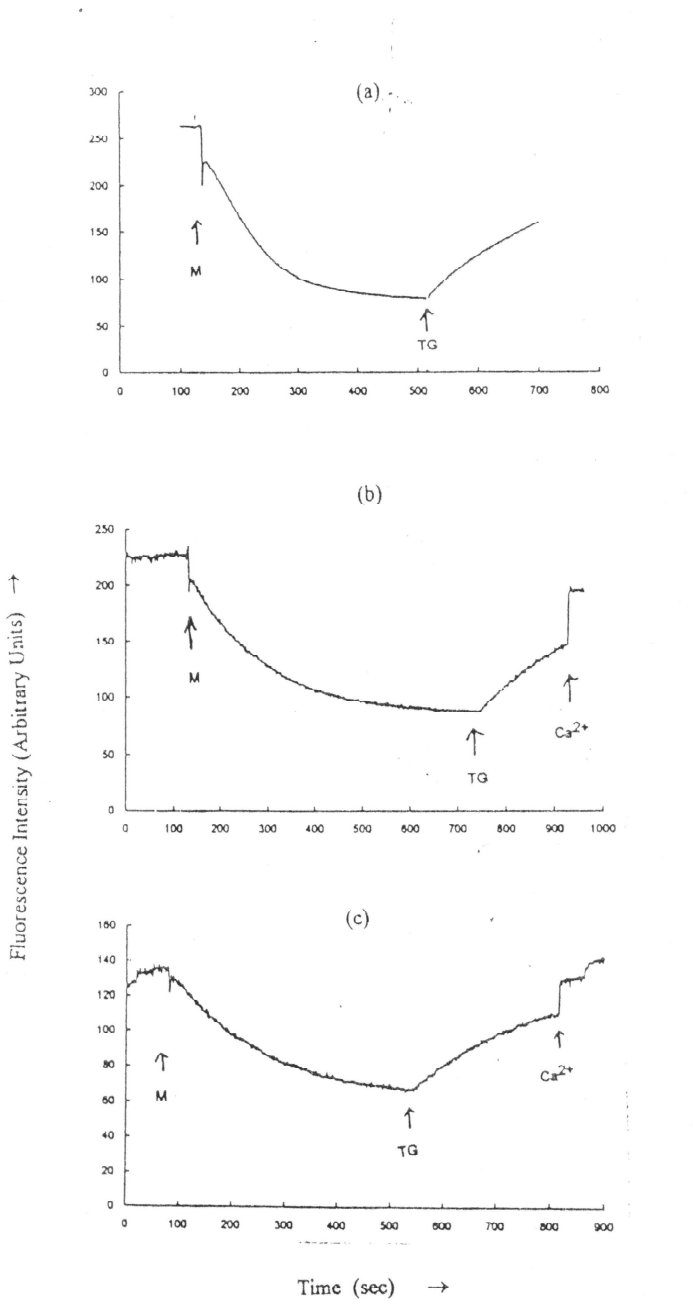

Fig. 3: Ionic requirements for thapsigargin-induced Ca^{2+} release from rat liver microsomal vesicles.

Microsomes (1.5 mg/ml) were incubated in (a) the basic reaction medium described in the "Materials and Methods" section, (b) a solution in which KCl and $MgCl_2$ in the basic medium were replaced with K-gluconate (50 mM) and Mg-gluconate (2 mM) respectively, (c) a solution in which $MgCl_2$ in the basic medium was replaced with Mg-gluconate (2 mM) and KCl was omitted without replacement. Arrows indicate where the following were added: M, microsomes; TG, thapsigargin (10 nM); Ca^{2+}, calcium ions (10 nmoles). Traces were obtained from data generated by Shimadzu RF 5000 software which were subsequently fed into Microsoft Excel software.

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