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Comparison of the effects of thapsigargin, 2,5-di-(tert-butyl)-1,4-hydroquinone and cyclopiazonic acid on calcium release from rat liver endoplasmic reticulum

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ABSTRACT: The effects of three novel intracellular Ca^{2+}-ATPase inhibitors on Ca^{2+} efflux from rat liver endoplasmic reticulum (ER) were compared. The three inhibitors: thapsigargin (TG); 2,5-di-(tert-butyl)-1,4-hydroquinone (DBHQ) and cyclopiazonic acid (CPA) released Ca^{2+} to various extents from the ER. Thapsigargin caused a slow but extensive release of Ca^{2+} from the ER, releasing almost all the Ca^{2+} accumulated by the vesicles during the uptake phase. The other two inhibitors did not release all the Ca^{2+} accumulated during the uptake phase. Furthermore, cyclopiazonic acid did not prevent the re-uptake of the Ca^{2+} added after the release process. These findings suggest that there may be some intracellular Ca^{2+} stores which are insensitive to DBHQ and cyclopiazonic acid.

Key Words: Ca^{2+} transport; Endoplasmic reticulum; Microsomes; Thapsigargin; 2,5-di-(tert-butyl)-1,4-hydroquinone; Cyclopiazonic acid.

INTRODUCTION

The regulation of intracellular Ca^{2+} concentration continues to attract widespread interest as a result of the important role of Ca^{2+} in mediating a wide variety of cellular processes (1-4). In the liver, cytosolic Ca^{2+} concentration is maintained at approximately 2 µM (2). This is achieved by the concerted activities of a plasma membrane Ca^{2+} pump which extrudes Ca^{2+} from the cytoplasmic compartment, and the active Ca^{2+} sequestration by mitochondria and endoplasmic reticulum (5-7). In muscle cells, the major reservoir of Ca^{2+} is the sarcoplasmic reticulum (SR). The release of Ca^{2+} from the SR causes contraction while the subsequent active transport of Ca^{2+} into the SR, through the activity of membrane-bound Ca^{2+}-ATPase, causes relaxation (1).

Quite recently, three new inhibitors of intracellular Ca^{2+}-ATPases were reported to elevate cytoplasmic Ca^{2+} in many cells by causing Ca^{2+} to be released from intracellular stores. These include the sesquiterpene lactone and tumour promoter thapsigargin (TG) (8-11), 2,5-di(tert-butyl)-1,4-hydroquinone (DBHQ) (12) and cyclopiazonic acid (CPA) (13). In the present study, we have compared the effects of these inhibitors on the release of Ca^{2+} from intracellular compartments such as the endoplasmic reticulum of rat liver.

MATERIALS AND METHODS

ATP (vanadate-free) and dithiothreitol were from Boehringer-Mannheim, UK Ltd. Thapsigargin, 2,5-di(tert-butyl)-1,4-hydroquinone, cyclopiazonic acid, 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 and Ca^{2+}

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uptake and release were monitored using the fluorescent Ca^{2+} indicator Fluo 3 as previously described (14). 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 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). Ca^{2+} release was also initiated by the addition of thapsigargin, DBHQ or CPA as described in the legend to Fig. 1. 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.

The protein concentrations of the microsomal fractions were determined by the procedure of Lowry *et al*. (15).

RESULTS AND DISCUSSION

It can be seen from Table 1a that thapsigargin causes a slow but extensive release of Ca^{2+} from rat liver endoplasmic reticulum, releasing almost all the Ca^{2+} accumulated during the uptake phase. With DBHQ and cyclopiazonic acid (Table 1 b and c), only a fraction (about 60 and 25 percent respectively) of the Ca^{2+} accumulated into the vesicles was released. Furthermore, cyclopiazonic acid did not prevent the re-uptake of the Ca^{2+} added to calibrate the system after the drug-induced Ca^{2+} release.

The results presented here show that while thapsigargin is able to release Ca^{2+} from most or all intracellular Ca^{2+} stores, some of these stores are insensitive to DBHQ and cyclopiazonic acid. Ca^{2+} uptake into microsomal vesicles is generally believed to be mediated by membrane-bound Ca^{2+}-ATPases (2,4,7). Inhibition of these ATPases has also been shown to cause Ca^{2+} release from the vesicles into the cytosol. However, the present results have shown that ER Ca^{2+}-ATPase is not very sensitive to inhibition by DBHQ and CPA. This view is further strengthened by the fact that there is a re-uptake of the Ca^{2+} added into the system after the limited Ca^{2+} release by CPA. This shows that the ATPase mediating the Ca^{2+} uptake into the vesicles is not totally inhibited by CPA.

The ER vesicles used in these experiments have been prepared by conventional techniques (14,16), hence the ATPase is expected to respond to classical inhibitors. It is quite possible that there may be some yet unidentified intracellular Ca^{2+} stores that are insensitive to DBHQ and CPA which may be responsibe for retaining the unreleased Ca^{2+} observed in these experiments.

It is very doubtful if the observed effects of DBHQ and CPA bear any relationship wih the phenomenon of quantal mobilization of Ca^{2+} stores which has been reported by several workers (17-20). This phenomenon has been observed in many cell types, including hepatocytes, when submaximal concentrations of inositol (1,4,5) trisphosphate [$Ins(1,4,5)P_3$] are used to release Ca^{2+} from intracellular stores.

Further experiments are needed to ascertain whether or not the partial emptying of Ca^{2+} stores by DBHQ or CPA is similar to the incremental responses induced by $Ins(1,4,5)P_3$.

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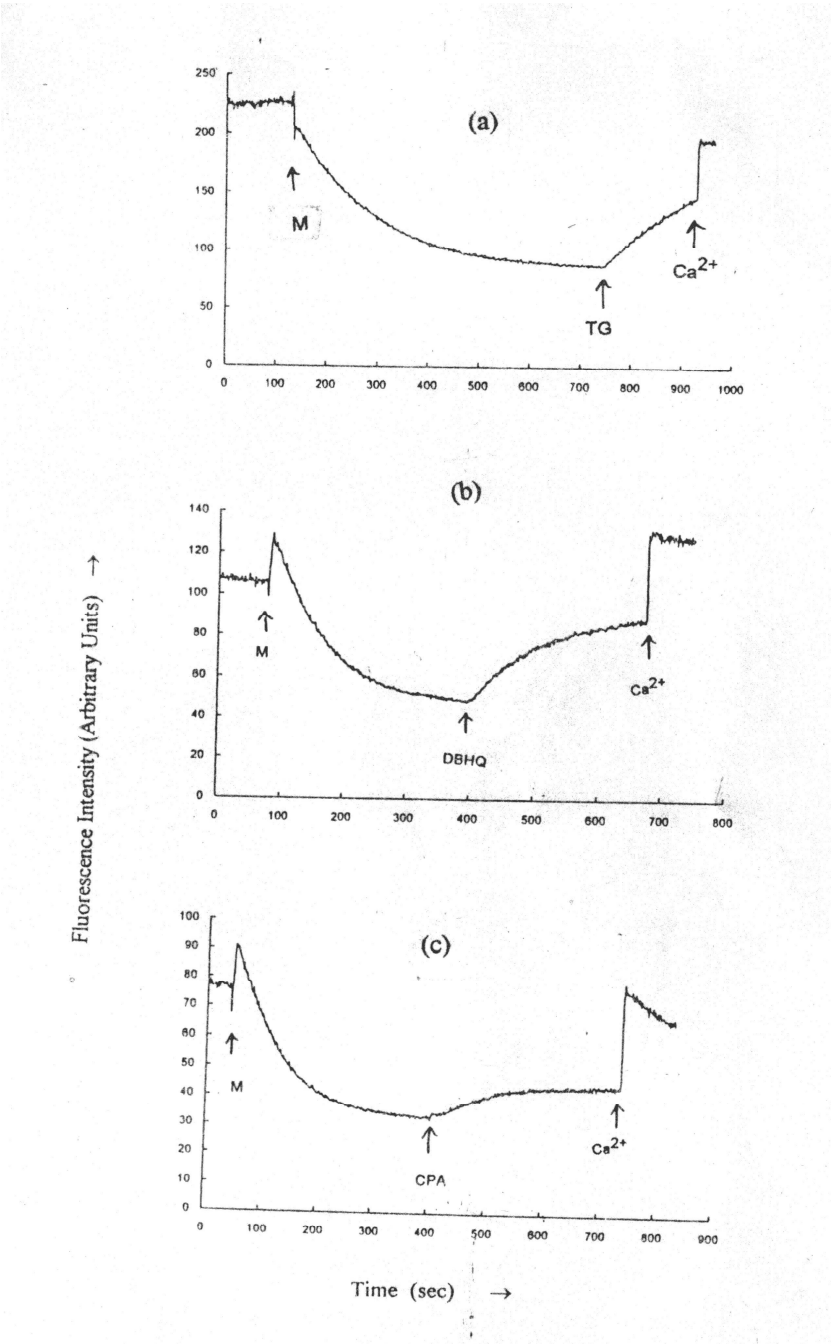

Fig. 1: Drug-induced Ca2+ release from rat liver endoplasmic reticulum.

Microsomes (1.5 mg/ml) were incubated in the basic medium described in the "Materials and Methods" section. Arrows indicate where the following were added: M, microsomes; TG, thapsigargin (100 nM); DBHQ, 2,5-di-(tert-butyl)-1,4-hydroquinone (2εM); CPA, cyclopiazonic acid (2εM); 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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