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cGMP-REGULATED STORE-OPERATED CALCIUM ENTRY IN HUMAN HEPATOMA CELLS

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This study aimed to investigate cGMP-regulated store-operated Ca^{2+} entry in human 7721 hepatoma cells. $[Ca^{2+}]_i$ was measured using Fura2/AM. After incubation of the cells with 4 μM thapsigargin, Ca^{2+} entry was evoked by application of 1 mM Ca^{2+} to extracellular solution and was blocked by 3 mM Ni^{2+}, indicating the presence of store-operated Ca^{2+} entry in human 7721 hepatoma cell line. Application of 8-Br-cGMP reduced the $[Ca^{2+}]_i$ in hepatoma 7721 cells by 80%. These data demonstrated for the first time that store-operated Ca^{2+} entry pathway is present in human hepatoma cells, which is regulated by cGMP.

KEYWORDS: store-operated Ca^{2+} entry; cGMP; hepatoma cell.

INTRODUCTION

Ion channels have been implicated in the invasion progress and metastasis of tumour cells and the ion channels involved are believed to be regulated by intracellular signalling molecules such as calcium (Wu, 1997; Bauer, 2000). Store-operated calcium entry is induced by intracellular calcium store depletion, and this calcium influx, negatively regulated by CGMP (Kwan, 2000; Yao, 2000), is known to stimulate production and release of NO by increasing nitric-oxide synthase activity (Bauer, 2000). Some studies have indicated that inhibitor of Ca^{2+}-mediated signal transduction can inhibit the production and release of NO, which has also been implicated in cancer metastasis *in vitro* and *in vivo*, and down-regulate matrix metaloproteinase-2 (MMP-2) and type IV collagenase (MMP-9) *in vivo* (Wu, 1997). Therefore, calcium-dependent signalling may be a new target for studies of growth and metastasis of cancer.

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By measuring $[Ca^{2+}]_i$, this study explored the presence the store-operated Ca^{2+} entry in hepatoma cells and its regulation by cGMP.

MATERIALS AND METHODS

Cell lines and reagents

The human 7721 hepatoma cell line was obtained from the Institute of Cell Biology, Academic Sinica. Cells were cultured in RPMI1640 medium (GIBCOBRL) supplemented with 10% FBS, 1% penicillin/streptomycin and 2% L-Glutamin, at 37°C and 5% CO_2. Furo2/AM was obtained from Molecular Probes, Inc. (Eugen, OR, U.S.A.), thapsigargin and 8-Br-cGMP were from Calbiochem (San Deigo, CA, U.S.A.). Other reagents are from Sigma Chemical (St Louis, MO, U.S.A.).

Measurement of intracellular free calcium concentration

Intracellular free calcium ($[Ca^{2+}]_i$) was measured using Fura-2 acetoxymethyl ester (Fura2/AM) (Negulescu, 1990; Ashton, 1993). Cells were loaded

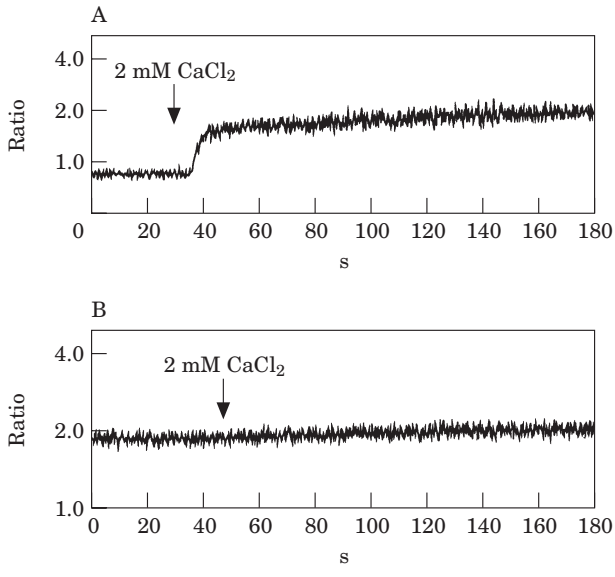

Fig. 1. Thapsigargin-induced rise in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was monitored in Fura2/AM-loaded human 7721 hepatoma cell line. Cells were $0Ca^{2+}$-PBS with 4 μM thapsigargin for 20 mn. Ni^{2+} were introduced 5 min prior to the experiments. At the time indicated by the arrow, the media were changed to the respective media containing 2 mM $CaCl_2$ without EGTA. A, thapsigargin; B, thapsigargin + 3 mM Ni^{2+}.

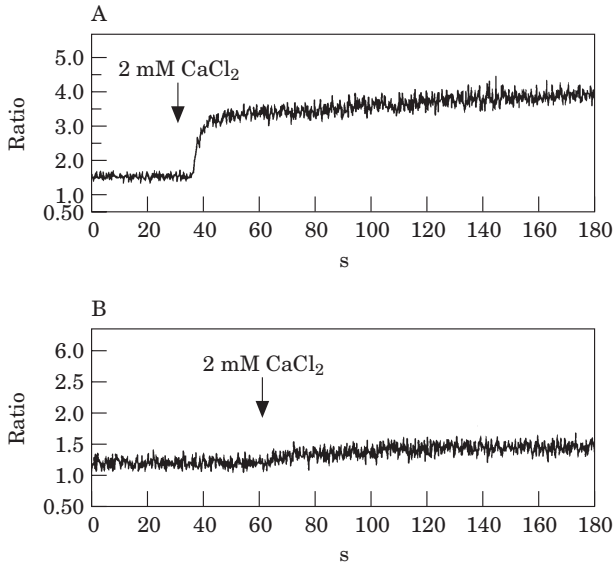

Fig. 2. Effects of 8-Br-cGMP on thapsigargin-induced rise in $[Ca^{2+}]_i$. Cells were $0Ca^{2+}$-PBS with 4 μM thapsigargin for 20 min. 8-Br-cGMP were introduced 5 min prior to the experiments. At the time indicated by the arrow, the media were changed to the respective media containing 2 mM $CaCl_2$ without EGTA. A, thapsigargin; B, thapsigargin + 2 mM 8-Br-cGMP.

with the dye by incubation with 3 μM Fura2/AM for 45 min in dark at 37°C in normal PBS (NPBS) containing 2 mM $CaCl_2$, pH 7.4. Cells were then washed and resuspended in NPBS. To start the experiment, cells were pretreated with 4 μM thapsigargin for 20 min. Then, the cells were washed with and maintained briefly in a medium PBS that contained no Ca^{2+} and 2 mM EGTA. Unless stated otherwise, the cells were pretreated with or without 8-Br-cGMP or $NiCl_2$ for 5 min. The fluorescent signal was monitored and recorded by an LS-50B luminescent spectrometer (Perkin Elmer, Germany). The excitation light at 340 nm and 380 nm was provided by a 150 W Xenon arc lamp (Perkin Elmer, Germany) and a filter wheel (Perkin Elmer, Germany) that containing 340 and 380 nm interference filters (Perkin Elmer, Germany). The emitted fluorescence at 510 nm was collected by a photomultiplier tube and recorded.

RESULTS AND DISCUSSION

Thapsigargin, a potent inhibitor of endoplasmic reticulum Ca^{2+}-ATPase, was used to deplete intracellular Ca^{2+} stores and induce calcium entry from extracellular space (Kwan, 2000). This elevation of intracellular $[Ca^{2+}]_i$ was evoked by application of Ca^{2+} to extracellular solution, and was blocked by Ni^{2+}. It suggests that the rise of $[Ca^{2+}]_i$ is caused by Ca^{2+} entry and this store-operated Ca^{2+} entry exists in human 7721 hepatoma cell line. 8-Br-cGMP is an activator of PKG. Application of 8-Br-cGMP reduced the $[Ca^{2+}]_i$ in a concentration-dependent manner. In hepatoma cell line 7721, 80% of the Ca^{2+} entry was inhibited by 8-Br-cGMP (2 mM). These data show that a store-operated Ca^{2+} entry pathway is present in human hepatoma cells and the store-operated Ca^{2+} entry sensitive to cGMP. The detailed signaling pathway involved and its precise role in metastasis of hepatoma cells remain to be elucidated. Further identification of the type of ion channel involved in the CGMP-regulated Ca^{2+} entry is currently undertaken.

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