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Depletion of Ca^{2+} from intracellular stores of the rat portal vein stimulates a tonic contraction

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Abstract

The possibility that Ca^{2+} store depletion can stimulate contraction of the rat portal vein was investigated in functional experiments. Ca^{2+} stores were depleted with phenylephrine or cyclopiazonic acid in the absence of extracellular Ca^{2+} and then washed out for 30 min. Upon re-addition of extracellular Ca^{2+}, a tonic contraction was produced, showing the stimulus for contraction was Ca^{2+} store depletion. The contractions were abolished by niflumic acid and nifedipine however, indicating they were dependent on depolarization resulting from opening of Ca^{2+}-activated Cl^- channels and Ca^{2+} influx through voltage-gated channels. Cumulative additions of phenylephrine below 3×10^{-6} M did not produce tonic contractions but did in high K^+ Krebs solution, where levcromakalim had no effect. This showed the tonic contractions were initially prevented by K^+ channel opening. Increased Ca^{2+} entry through voltage-gated channels may therefore stimulate Ca^{2+}-activated Cl^- channels. Ca^{2+} store depletion could stimulate this by opening store-operated non-selective cation channels, resulting in depolarization.

Keywords: Rat portal vein; Capacitative Ca^{2+} entry; Store operated Ca^{2+} entry; Cyclopiazonic acid; Niflumic acid

1. Introduction

Depletion of Ca^{2+} from intracellular stores by inositol 1,4,5-trisphosphate (IP_3) generating agonists stimulates extracellular Ca^{2+} influx through non-voltage-gated channels in many cell types, known as capacitative or store-operated Ca^{2+} influx (Parekh and Penner, 1997; Putney, 1986). This can also be stimulated by cyclopiazonic acid, an inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase (Seidler et al., 1989). Capacitative Ca^{2+} influx was first shown in non-excitable cells to be mediated via Ca^{2+} selective channels (Hoth and Penner, 1992) and then through non-selective cation channels in endothelial cells (Zhang et al., 1994). In smooth muscle Ca^{2+} store depletion also stimulates non-selective cation channels (Trepakova et al., 2001; Wayman et al., 1996) including rabbit portal vein (Albert and Large, 2002). Capacitative Ca^{2+} influx in smooth muscle has been shown to result in a tonic contraction in tissues such as rat spleen (Burt et al., 1995) rat ileum (Ohta et al., 1995) mouse anococcygeus (Wayman et al., 1996) and rat pulmonary artey (McDaniel et al., 2001; Ng and Gurney, 2001). It has been proposed that some members of the transient receptor potential (*trp*) gene family encode for the cation channels activated by Ca^{2+} store depletion (for reviews see Hofmann et al., 2000; Montell et al., 2002).

The rat isolated portal vein displays spontaneous phasic contractions. These are dependent on depolarization and influx of Ca^{2+} through voltage-gated channels which stimulates release of Ca^{2+} from ryanodine stores (Burt, 2003; Grégoire et al., 1993). Stimulation of α_1-adrenoceptors in rat portal vein cells has been shown to produce a rise in IP_3 (Leprêtre et al., 1994a). In functional studies using the rat isolated portal vein it has been shown that depletion of Ca^{2+} from intracellular stores via α_1-adrenoceptor stimulation or by cyclopiazonic acid potentiated the spontaneous

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contractions without increasing resting tone (Burt, 2004). Ca^{2+} store depletion has been shown to stimulate Ca^{2+} influx (Pacaud et al., 1993) and non-selective cation channels (Albert and Large, 2002) in portal vein myocytes. Stimulation of non-selective cation channels by Ca^{2+} store depletion can also result in depolarization (Scharff and Foder, 1996). It is possible that depolarization of the portal vein stimulated in this way potentiates depolarizations associated with the spontaneous contractions. The results of this study show that greater depletion of Ca^{2+} from intracellular stores of the rat portal vein than required to potentiate the spontaneous contractions can also stimulate a tonic contraction. This involves depolarization via opening of Ca^{2+}-activated Cl^- channels, which may be stimulated by increased Ca^{2+} entry through voltage-gated channels.

2. Methods

All experimental protocols were approved by the institutional ethics committee. Male Sprague–Dawley rats between 350 and 450 g were stunned and killed by cervical dislocation. The portal vein was removed into Krebs solution (Krebs) and associated connective tissue was dissected away. The tissues (10–15 mm) were suspended longitudinally in 5 ml tissue baths containing Krebs solution of the following composition (mM): Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128, HCO_3^- 25, HPO_4^{2-} 1.2, SO_4^{2-} 1.2 and glucose 11, at 25 °C and bubbled with 95% O_2/5% CO_2. A modified high K^+ Krebs was sometimes used of the same composition except for an increase in K^+ to 50 mM and an equivalent decrease in Na^+ to 98.9 mM. When Ca^{2+} free Krebs was used this always contained EGTA (1 mM) unless otherwise stated. The portal veins were placed under 0.5 g resting tension and equilibrated for 1 h. Changes in isometric tension were measured using Grass FT.03 transducers and recorded by Biopac Systems Inc. MP100WS for Windows.

Experiments were carried out at 25 °C so that the results were consistent with Burt (2003, 2004). Preliminary experiments have shown contractions of this tissue to be similar at 25 °C and 37 °C. Phenylephrine (10^{-4} M, which produced a maximum response) was added to all tissues initially, with a recovery period of 45 min following washout. Drugs were incubated for 30 min with tissues unless otherwise stated.

2.1. Experiments in normal Krebs

The response to a single high concentration of phenylephrine (10^{-4} M) and to cumulative additions of phenylephrine (10^{-8} M–3×10^{-5} M) was recorded. The effect of the protein kinase C (PKC) inhibitor calphostin C (10^{-6} M, 1 h incubation) or the K^+ channel opener levcromakalim (3×10^{-6} M) was measured on the tonic contraction to phenylephrine (10^{-6} M).

2.1.1. The effect of depleting intracellular Ca^{2+} stores in Ca^{2+}-free Krebs

These experiments were designed to show if the stimulus for the tonic contraction depended on the intracellular Ca^{2+} stores being depleted rather than the presence of the depleting agent. Phenylephrine (10^{-4} M) or cyclopiazonic acid (10^{-5} M) was added to tissues in Ca^{2+}-free Krebs for 10 or 15 min, respectively. They were then washed out for 30 min, still in Ca^{2+}-free Krebs, which was then changed to Ca^{2+}-free Krebs without EGTA. Total time in Ca^{2+}-free Krebs was 45 min. Ca^{2+} (2.5 mM) was then added to the tissues and responses following this measured. Control tissues were treated in the same way except no phenylephrine or cyclopiazonic acid was added. For some tissues, following the washout of phenylephrine or cyclopiazonic acid, nifedipine (3×10^{-7} M) or niflumic acid (3×10^{-5} M) was added before the addition of Ca^{2+} (2.5 mM).

2.2. Experiments in high K^+ Krebs

These experiments were designed to show the possible effect of K^+ channels on contractions of the portal vein. It has been shown previously that high K^+ Krebs can abolish the effect of K^+ channel opening (Cook et al., 1988). Following a contraction to phenylephrine (10^{-4} M) in normal Krebs, some tissues were placed in the modified high K^+ (50 mM) Krebs. Experiments were then performed once the contraction to high K^+ Krebs had returned to baseline or close to it (after about 40 min).

The effect of the K^+ channel opener levcromakalim (3×10^{-6} M) was measured on the response to phenylephrine (10^{-6} M). The effect of the K^+ channel blocker tetraethylammonium (10^{-3} M–3×10^{-3} M) was also measured in high K^+ Krebs.

A cumulative concentration–response curve to phenylephrine was recorded and the effect of nifedipine (3×10^{-7} M) or niflumic acid (3×10^{-5} M) measured on this. The response to cyclopiazonic acid (10^{-5} M) was recorded and the effect of removing extracellular Ca^{2+} or niflumic acid (3×10^{-5} M) on this response was measured.

2.2.1. The effect of depleting intracellular Ca^{2+} stores in high K^+, Ca^{2+}-free Krebs

Phenylephrine (10^{-4} M) was added to tissues in high K^+, Ca^{2+}-free Krebs for 5 min. It was then washed out for 30 min, still in high K^+, Ca^{2+}-free Krebs, which was then changed to high K^+, Ca^{2+}-free Krebs without EGTA. Ca^{2+} (2.5 mM) was then added to the tissues and the following response measured. Control tissues were treated in the same way except no phenylephrine was added.

2.3. Data analysis

All contractions were measured as a percentage response of that to phenylephrine (10^{-4} M) and calculated as the mean from 4 separate experiments (n=4) unless otherwise