Evidence that 2-aminoethyl diphenylborate is a novel inhibitor of store-operated Ca^{2+} channels in liver cells, and acts through a mechanism which does not involve inositol trisphosphate receptors

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The compound 2-aminoethyl diphenylborate (2-APB), an inhibitor of Ins(1,4,5)P_3 receptor action in some cell types, has been used to assess the role of Ins(1,4,5)P_3 receptors in the activation of store-operated Ca^{2+} channels (SOCs) [Ma, Patterson, van Rossum, Birnbaumer, Mikoshiba and Gill (2000) Science **287**, 1647–1651]. In freshly-isolated rat hepatocytes, 2-APB inhibited thapsigargin- and vasopressin-stimulated Ca^{2+} inflow (measured using fura-2) with no detectable effect on the release of Ca^{2+} from intracellular stores. The concentration of 2-APB which gave half-maximal inhibition of Ca^{2+} inflow was approx. 10 μM. 2-APB also inhibited Ca^{2+} inflow initiated by a low concentration of adenophostin A but had no effect on maitotoxin-stimulated Ca^{2+} inflow through non-selective cation channels. The onset of the inhibitory effect of 2-APB on thapsigargin-stimulated Ca^{2+} inflow was rapid. When 2-APB was added to rat hepatocytes in the presence of extracellular Ca^{2+} after a vasopressin-induced plateau in the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) had been established, the kinetics of the decrease in $[Ca^{2+}]_{cyt}$ were identical with those induced by the addition of 50 μM Gd^{3+} (gadolinium). 2-APB did not inhibit the release of Ca^{2+} from intracellular stores induced by the addition of Ins(1,4,5)P_3 to permeabilized hepatocytes. In the H4-IIE rat hepatoma cell line, 2-APB inhibited thapsigargin-stimulated Ca^{2+} inflow (measured using fura-2) and, in whole-cell patch-clamp experiments, the Ins(1,4,5)P_3-induced inward current carried by Ca^{2+}. It was concluded that, in liver cells, 2-APB inhibited SOCs through a mechanism which involved the binding of 2-APB to either the channel protein or an associated regulatory protein. 2-APB appeared to be a novel inhibitor of SOCs in liver cells with a mechanism of action which, in this cell type, is unlikely to involve an interaction of 2-APB with Ins(1,4,5)P_3 receptors. The need for caution in the use of 2-APB as a probe for the involvement of Ins(1,4,5)P_3 receptors in the activation of SOCs in other cell types is briefly discussed.

Key words: gadolinium (Gd^{3+}), H4-IIE cells, maitotoxin, patch-clamp recording, rat hepatocytes.

INTRODUCTION

Store-operated Ca^{2+} channels (SOCs) are defined as plasma membrane Ca^{2+} channels, the activation of which is mediated by a decrease in Ca^{2+} in the endoplasmic reticulum (ER) (reviewed in [1–3]). In liver cells and in other animal cells, SOCs are thought to be responsible for replenishing Ca^{2+} lost from the ER (via the cytoplasmic space and plasma membrane Ca^{2+}/Mg^{2+} ATPase) during agonist-induced increases in the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) [1–4]. The structures and mechanism of activation of SOCs are not well understood [1–3]. Recent work in our laboratory has provided evidence that a subset of Ins(1,4,5)P_3 receptors, possibly type 1 Ins(1,4,5)P_3 receptors, is required for the activation of SOCs in liver cells [5]. The Ins(1,4,5)P_3 receptors involved in SOC activation are characterized by a relatively high sensitivity to adenophostin A and Ins(1,4,5)P_3. Moreover, it has also been shown that SOCs in liver cells can be activated by a relatively small decrease in the concentration of Ca^{2+} in the ER [5]. Other experiments have provided evidence that the activation of rat hepatocyte SOCs requires the trimeric G-protein, G_{i2}, F-actin, the integrity of the ER and/or the location of a region of the ER near the plasma membrane [6,7].

Recent experiments conducted with human embryonic kidney (HEK-293) and DDT_1-MF2 smooth muscle cells have employed the compound 2-aminoethyl diphenylborate (2-APB) to elucidate the role of Ins(1,4,5)P_3 receptors in the activation of plasma membrane Ca^{2+} channels [8,9]. 2-APB, which is membrane permeable, has been shown to inhibit Ins(1,4,5)P_3-induced Ca^{2+} release from isolated microsomes and from the ER in intact cells [10,11]. There is some evidence to indicate that 2-APB inhibits Ins(1,4,5)P_3 receptor function by binding at a site on the Ins(1,4,5)P_3 receptor protein that is separate from the Ins(1,4,5)P_3 binding site [10]. The results of studies on the actions of 2-APB on Ca^{2+} inflow to HEK-293 and smooth muscle cells led to the conclusion that some Ins(1,4,5)P_3 receptor proteins are required in the process by which a decrease in ER Ca^{2+} concentration activates SOCs [8,9].

The aim of the present experiments was to use 2-APB as a probe to further elucidate the role of Ins(1,4,5)P_3 receptors in the activation of SOCs in liver cells. The results provide evidence which indicates that, in this cell type, 2-APB inhibits SOCs by interacting with the SOC protein or with an associated regulatory protein. In contrast with the effects of 2-APB on HEK-293 and smooth muscle cells [8,9], 2-APB did not readily inhibit Ins(1,4,5)P_3-induced release of Ca^{2+} from the ER in either intact or permeabilized liver cells.

MATERIALS AND METHODS

Materials

2-APB, Ruthenium Red and maitotoxin were obtained from Sigma-Aldrich (Castle Hill, N.S.W., Australia). 2-APB was

Abbreviations used: 2-APB, 2-aminoethyl diphenylborate; SOC, store-operated Ca^{2+} channel; ER, endoplasmic reticulum; $[Ca^{2+}]_{cyt}$, cytoplasmic free Ca^{2+} concentration.

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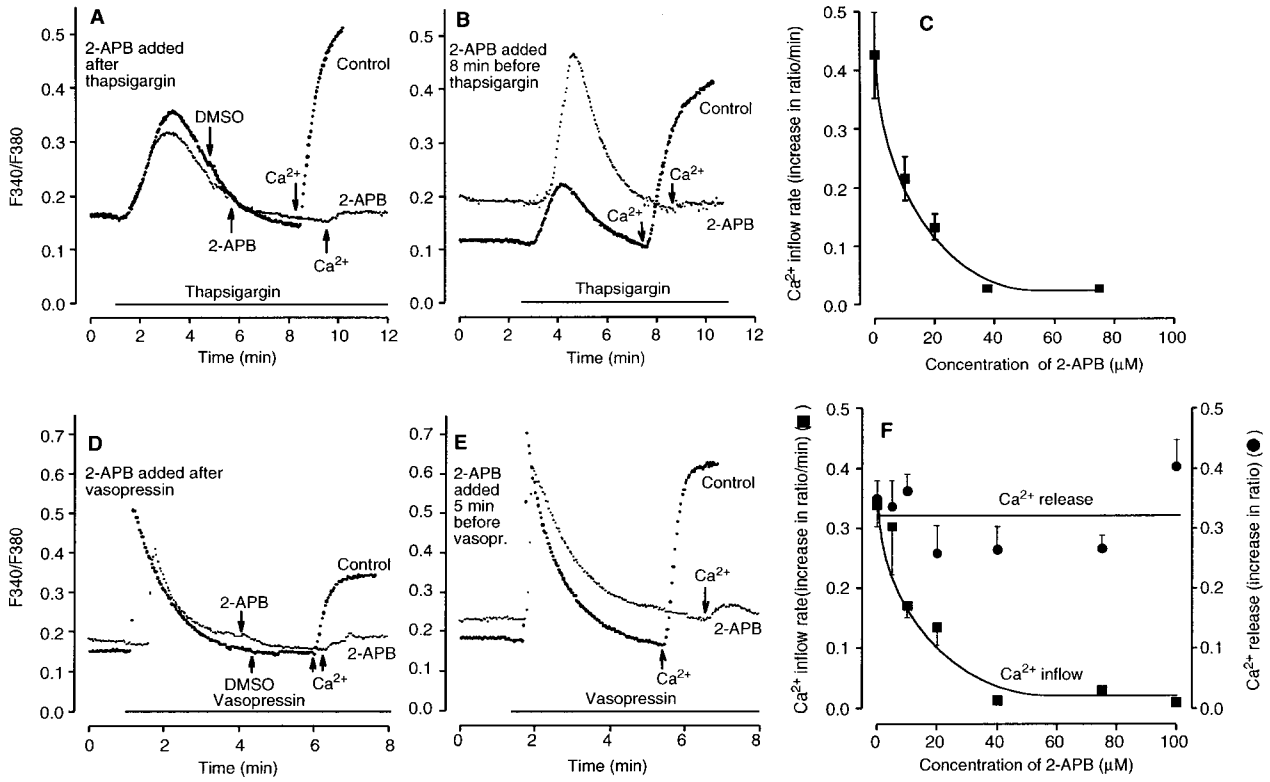

Figure 1 Inhibition by 2-APB of thapsigargin- (A–C) and vasopressin- (D, E) induced Ca^{2+} inflow in freshly-isolated rat hepatocytes

(**A**, **B**, **D**, **E**) Freshly-isolated rat hepatocytes loaded with fura-2 were incubated initially in the absence of added extracellular Ca^{2+}. The times of addition of thapsigargin (10 μM) or vasopressin (40 nM) are indicated by the horizontal bars, and the times of addition of 2-APB (75 μM) or 1% (v/v, final concentration) DMSO or $CaCl_2$ (1.5 mM) are indicated by arrows. Each trace is the mean of the results obtained for 5–8 (**A** and **B**) or 4–9 (**D** and **E**) cells. (**C**, **F**) Concentration-response curves for the effects of 2-APB on thapsigargin- and vasopressin-induced Ca^{2+} inflow and release. The results are the means $\pm$ S.E.M. of the values obtained for 5–8 cells (**C**) or 15–56 cells (**F**) from 2–5 separate cell preparations.

dissolved in DMSO [final concentration in the incubation mixture was $\leqslant 1\%$ (v/v)]. Fluo-3, fura-2 and fura-2 acetoxymethyl ester were purchased from Molecular Probes (Eugene, OR, U.S.A.), and Ins(1,4,5)P_3 was obtained from Calbiochem-Novabiochem (Alexandria, N.S.W., Australia). Digitonin was from ICN Biomedicals (Seven Hills, N.S.W. Australia) and phosphocreatine and creatine kinase (EC 2.7.3.2) were purchased from Boehringer-Mannheim (Castle Hill, N.S.W., Australia). Other reagents were obtained from sources described previously [5,7].

Methods

The preparation of freshly-isolated rat hepatocytes, attachment of the cells to collagen-coated coverslips, and loading of the cytoplasmic space with fura-2 by microinjection, were conducted as described previously [5,7]. H4-IIE cells (derived from the rat Reuber hepatoma) were cultured on collagen-coated coverslips as described previously [12]. H4-IIE cells were loaded with fura-2 by incubation with 10 μM fura-2 acetoxymethyl ester and 0.025% (w/v) pluronic acid in Dulbecco's modified Eagle's medium for 30 min at 37 °C. The coverslips were then washed with Ca^{2+}-free Hanks medium. More than 80% of the fura-2 was shown to be located in the cytoplasmic space. The fluorescence of cytoplasmic fura-2 in individual rat hepatocytes and H4-IIE cells was measured as described previously [7].

The permeabilization of hepatocytes in suspension and subsequent measurement of Ins(1,4,5)P_3-induced release of Ca^{2+} were based on established procedures [13]. Hepatocytes (approx. 2×10^6 cells/ml) were washed in 'intracellular medium' [120 mM KCl, 10 mM NaCl, 20 mM KH_2PO_4, 20 mM Hepes (adjusted to pH 7.2 with Tris base), which was treated with Chelex-100 to remove Ca^{2+} ions] and resuspended in intracellular medium containing antipain (1 μg/ml), pepstatin (1μg/ml), leupeptin (1 μg/ml), 1 μM Ruthenium Red and 1 μM fluo-3. The fluorescence of the stirred suspension of cells was monitored at 37 °C in a Perkin-Elmer LS50B spectrofluorimeter with excitation and emission wavelengths of 503 nm and 530 nm respectively. Incubation for 3 min following the addition of 40 μM digitonin resulted in essentially 100% permeabilization of the cells, as judged by Trypan Blue staining. A mixture of $MgCl_2$ (2 mM), ATP (2 mM), phosphocreatine (5 mM) and creatine kinase (5 units/ml) was then added to promote loading of cellular stores with Ca^{2+}. The release of Ca^{2+} was subsequently induced by the addition of 5 μM Ins(1,4,5)P_3. The concentration of free Ca^{2+} in the suspension before the addition of Ins(1,4,5)P_3 was 100–200 nM.

Whole-cell patch clamping [14] of H4-IIE cells was carried out at room temperature using a computer-based patch-clamp amplifier (EPC-9; HEKA Electronics, Lambrecht, Germany) and PULSE software (HEKA Electronics). The bath solution contained 140 mM NaCl, 4 mM CsCl, 10 mM $CaCl_2$, 2 mM $MgCl_2$ and 10 mM Hepes, adjusted to pH 7.4 with NaOH. The standard internal solution (high Ca^{2+} buffer internal solution) contained 120 mM Cs glutamate, 10 mM CsCl, 10 mM EGTA and 10 mM Hepes, adjusted to pH 7.2 with KOH. Depletion of intracellular

Ca^{2+} stores was achieved by adding 20 μM Ins(1,4,5)P_3 to the internal solution. Patch pipettes were pulled from borosilicate glass, coated with Sylgard and fire-polished. Pipette resistance was 2–4 MΩ. In order to monitor the development of the Ca^{2+} release-activated Ca^{2+} current, voltage ramps between -120 and $+60$ mV were applied every 2 s, starting immediately after achieving the whole-cell configuration. Early traces were used for leakage subtraction. Acquired currents were filtered at 2.7 kHz and sampled at 10 kHz. Traces were further digitally filtered at 1.5 kHz. All voltages shown are nominal voltages and have not been corrected for the liquid junction potential of -18 mV between the bath and electrode solutions (estimated using the JPCalc programme [15]). The holding potential was 0 mV throughout. Compensation for cell capacitance and series resistance was made automatically by the EPC9 amplifier before each voltage ramp.

RESULTS

For the following results: Ca^{2+} concentrations are given as the ratio of fluorescence obtained at the excitation wavelengths 340 nm and 380 nm (F340/F380); resting levels of Ca^{2+} in hepatocytes and H4-IIE cells were 30–100 nM; and, unless indicated otherwise, the results are presented as the means $\pm$ S.E.M. with the number of cells tested shown in parentheses.

At a concentration of 75 μM, 2-APB completely inhibited thapsigargin-stimulated Ca^{2+} inflow when added either after or before thapsigargin (Figures 1A and 1B). This concentration of 2-APB did not inhibit the ability of thapsigargin to release Ca^{2+} from intracellular stores. In the presence or absence of 75 μM 2-APB, the amount of Ca^{2+} released by thapsigargin was 0.30 ± 0.07 (8) and 0.11 ± 0.02 (8) fluorescence ratio units respectively. The concentration of 2-APB which gave half-maximal inhibition of thapsigargin-stimulated Ca^{2+} inflow was about 10 μM (Figure 1C).

In an experiment similar to that shown in Figure 1(A), the time of onset of the inhibitory action of 2-APB on thapsigargin-stimulated Ca^{2+} inflow was investigated by adding the compound at various times before the addition of 1.5 mM extracellular Ca^{2+}. The rates of Ca^{2+} inflow following the addition of 1.5 mM Ca^{2+} alone, a mixture of 2-APB (75 μM) and Ca^{2+}, 2-APB at 30 s before Ca^{2+} addition, and 2-APB at 14 min before Ca^{2+} addition were: 0.476 ± 0.075 (19), 0.162 ± 0.025 (18), 0.050 ± 0.022 (10) and 0.015 ± 0.003 (8) fluorescence ratio units/min respectively. The observations that the simultaneous addition of 2-APB and Ca^{2+} caused 65% inhibition of thapsigargin-stimulated Ca^{2+} inflow, and the addition of 2-APB 30 s before Ca^{2+} caused almost complete inhibition of Ca^{2+} inflow, indicated that onset of the inhibition by 2-APB was rapid.

2-APB inhibited vasopressin-stimulated Ca^{2+} inflow (Figures 1D and 1E) in a manner similar to that observed for its effect on thapsigargin-stimulated Ca^{2+} inflow. The concentration of 2-APB which gave half-maximal inhibition of vasopressin-stimulated Ca^{2+} inflow was about 10 μM (Figure 1F). At concentrations up to 100 μM, 2-APB had essentially no effect on vasopressin-induced Ca^{2+} release from intracellular stores (Figure 1F). An increase in the time of preincubation of the cells with 75 μM 2-APB from 5 (compare with Figure 1E) to 25 min before the addition of vasopressin did not cause a detectable inhibition of vasopressin-induced Ca^{2+} release from intracellular stores, but completely inhibited vasopressin-stimulated Ca^{2+} inflow (results not shown). In order to test the effect of 2-APB on the release of Ca^{2+} from intracellular stores more directly, hepatocytes were permeabilized with digitonin, the stores refilled with Ca^{2+} by the provision of ATP, and the effect of 2-APB on Ins(1,4,5)P_3-induced Ca^{2+} release was measured. 2-APB (75 μM) did not inhibit Ins(1,4,5)P_3-induced release of Ca^{2+} (Figure 2). The amounts of Ca^{2+} released (nmol) in the presence or absence of 2-APB were 35.8 ± 2.0 (4) and 33.3 ± 0.9 (3) respectively.

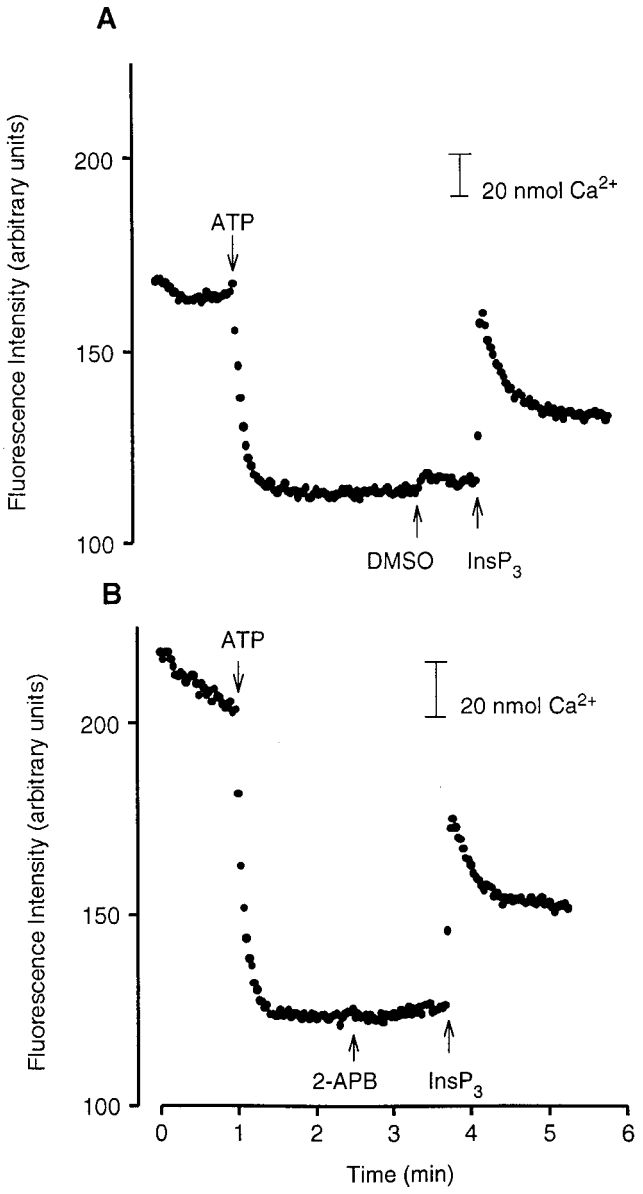

Figure 2 2-APB does not inhibit Ins(1,4,5)P_3-induced Ca^{2+} release from intracellular stores in permeabilized hepatocytes

Freshly-isolated rat hepatocytes, suspended in intracellular medium which contained protease inhibitors, 1 μM fluo-3 and 1 μM Ruthenium Red, were permeabilized with digitonin as described in the Materials and methods section. The times of addition of ATP (2 mM $MgCl_2$, 2 mM ATP, 5 mM phosphocreatine and 5 units/ml creatine kinase), 1% (v/v) DMSO, 75 μM 2-APB and 5 μM Ins(1,4,5)P_3 ($InsP_3$) are indicated with arrows. The calibration bars in the upper right corner of each panel represent the net change in fluorescence upon addition of 20 nmol Ca^{2+}. The traces shown are representative of those obtained from eight similar experiments conducted using three separate preparations of hepatocytes. Similar results were obtained when Ruthenium Red was replaced with a mixture of rotenone (1 μg/ml), oligomycin (5 μg/ml) and 5 μM carbonyl cyanide *m*-chlorophenylhydrazone (results not shown).

The results obtained with thapsigargin and vasopressin indicated that concentrations of 2-APB in the range 5–100 μM

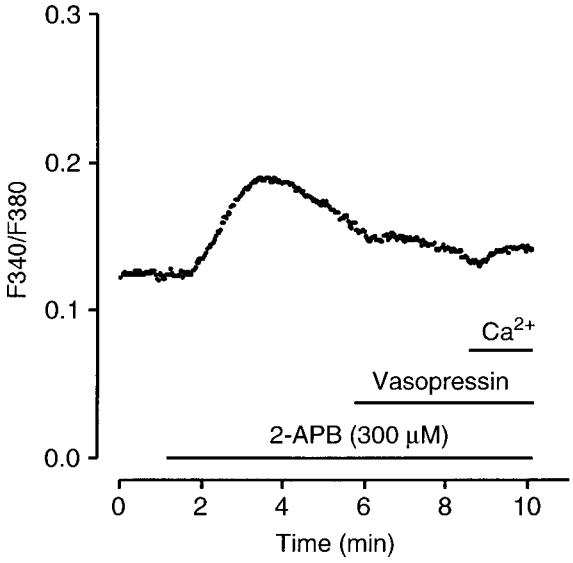

Figure 3 Release of Ca^{2+} from intracellular stores is induced by a high concentration of 2-APB (300 μM) in the absence of an agonist

Freshly-isolated rat hepatocytes loaded with fura-2 were incubated initially in the absence of added extracellular Ca^{2+}. The times of addition of vasopressin (40 nM), 2-APB (300 μM) and $CaCl_2$ (1.5 mM) are indicated by the horizontal bars. The trace shown is the mean of the results obtained for nine cells.

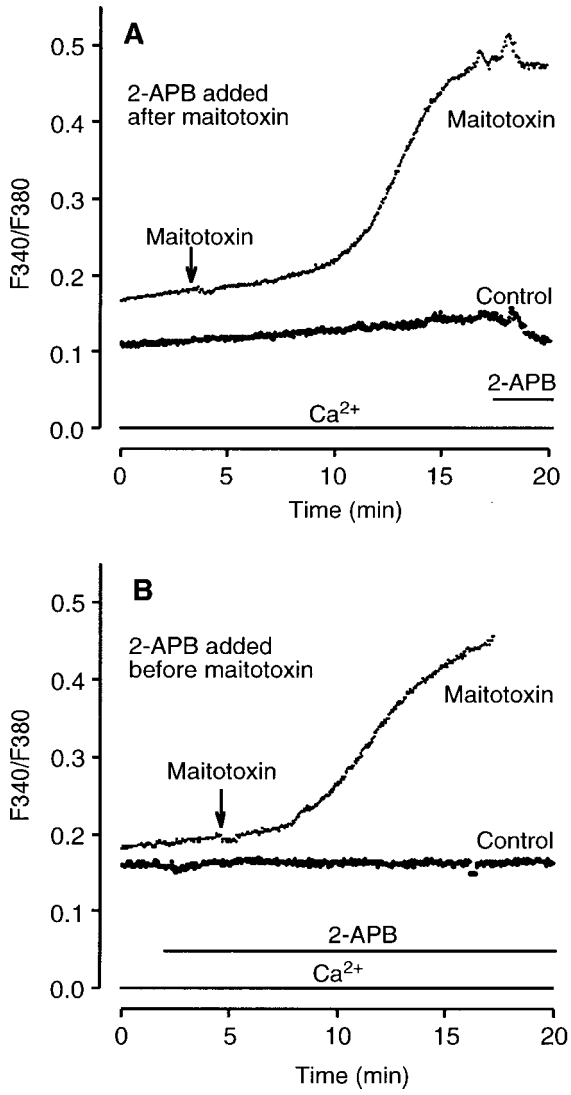

Figure 4 2-APB does not inhibit maitotoxin-induced Ca^{2+} inflow in rat hepatocytes

Freshly-isolated rat hepatocytes loaded with fura-2 were incubated in the presence of 1.5 mM Ca^{2+}. The times of addition of 2-APB (100 μM) and $CaCl_2$ (1.5 mM) are indicated by the horizontal bars, and the times of addition of maitotoxin (300 pM) by arrows. Each trace is the mean of the results obtained for 5–10 cells.

(compared with 75 μM, which completely inhibited Ca^{2+} inflow) did not affect the release of Ca^{2+} from intracellular stores. A substantially higher concentration of 2-APB (300 μM) induced the release of Ca^{2+} from intracellular stores in the absence of an agonist, and abolished the ability of vasopressin to release Ca^{2+} (Figure 3).

2-APB completely inhibited Ca^{2+} inflow initiated by adenophostin A [5,16] when 2-APB (75 μM) was added either before or after the introduction of adenophostin A to the cytoplasmic space (estimated intracellular concentration about 25 nM, administered by microinjection). The rates of adenophostin A-induced Ca^{2+} inflow were 0.021 ± 0.002 (37) and 0.193 ± 0.028 (18) fluorescence ratio units/min in the presence and absence of 2-APB, respectively (results not shown).

Maitotoxin has been shown to activate Ca^{2+} inflow to liver and other cells through non-selective cation channels [17–20]. 2-APB (100 μM) did not inhibit maitotoxin-induced Ca^{2+} inflow to rat hepatocytes (as assessed by the maitotoxin-induced increase in $[Ca^{2+}]_{cyt}$) when 2-APB was added either after the maitotoxin-induced plateau in $[Ca^{2+}]_{cyt}$ had been reached (Figure 4A) or before the addition of maitotoxin (Figure 4B).

The ability of 2-APB to inhibit vasopressin-stimulated Ca^{2+} inflow, once this had been activated, was compared with that of Gd^{3+}, a potent inhibitor of SOCs in hepatocytes [21]. Each inhibitor was added to cells that had been incubated in the presence of 1.5 mM Ca^{2+} after the addition of vasopressin and after the plateau of increased $[Ca^{2+}]_{cyt}$ had been established (Figure 5). {It has been shown previously that the plateau (sustained increase in $[Ca^{2+}]_{cyt}$) is due to enhanced Ca^{2+} inflow [1,3].} The time course for the decrease in $[Ca^{2+}]_{cyt}$ induced by 2-APB was similar to that for the decrease in $[Ca^{2+}]_{cyt}$ induced by the addition of 50 μM Gd^{3+} (Figure 5). For 2-APB and Gd^{3+}, the initial rates of decrease in $[Ca^{2+}]_{cyt}$ were 0.130 ± 0.014 (10) and 0.114 ± 0.013 (8) fluorescence ratio units/min, and the half times of the decrease in $[Ca^{2+}]_{cyt}$ were 2.5 ± 0.2 (10) and 2.6 ± 0.2 (8) min respectively.

It has been shown previously that H4-IIE cells express SOCs [12] with biophysical properties indistinguishable from those of the Ca^{2+}-release-activated Ca^{2+} channels detected in lymphocytes and mast cells (G. Rychkov, H. M. Brereton, M. L. Harland and G. J. Barritt, unpublished work). 2-APB (100 μM) inhibited thapsigargin-stimulated Ca^{2+} inflow to H4-IIE cells measured using fura-2 (Figure 6A). In whole-cell patch-clamp experiments, 2-APB (50 μM) completely inhibited the inward current induced by perfusion of H4-IIE cells with Ins(1,4,5)P_3 (Figure 6B and insert). This inhibition was rapid in onset and reversed by the removal of 2-APB from the external medium, and subsequent perfusion of the cells with buffer (Figure 6B). Reversal of 70% of the inhibitory effect of 2-APB required 3 min.

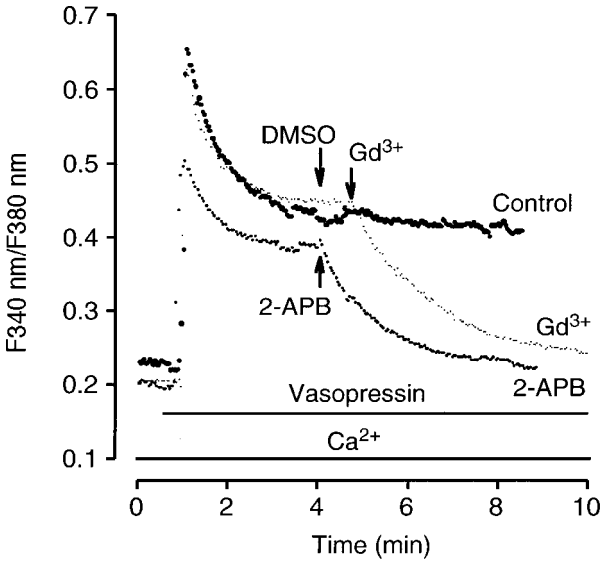

Figure 5 Comparison of the effects of 2-APB and Gd^{3+} on the cytoplasmic free Ca^{2+} concentration elevated by the addition of vasopressin to cells incubated in the presence of extracellular Ca^{2+}

Freshly-isolated rat hepatocytes loaded with fura-2 were incubated in the presence of 1.5 mM Ca^{2+}. The times of addition of vasopressin (40 nM) and $CaCl_2$ (1.5 mM) are indicated by the horizontal bars and of 2-APB (75 μM), DMSO [0.1% (v/v)], and $GdCl_2$ (50 μM) by arrows. Each trace is the mean of the results obtained for 5–10 cells.

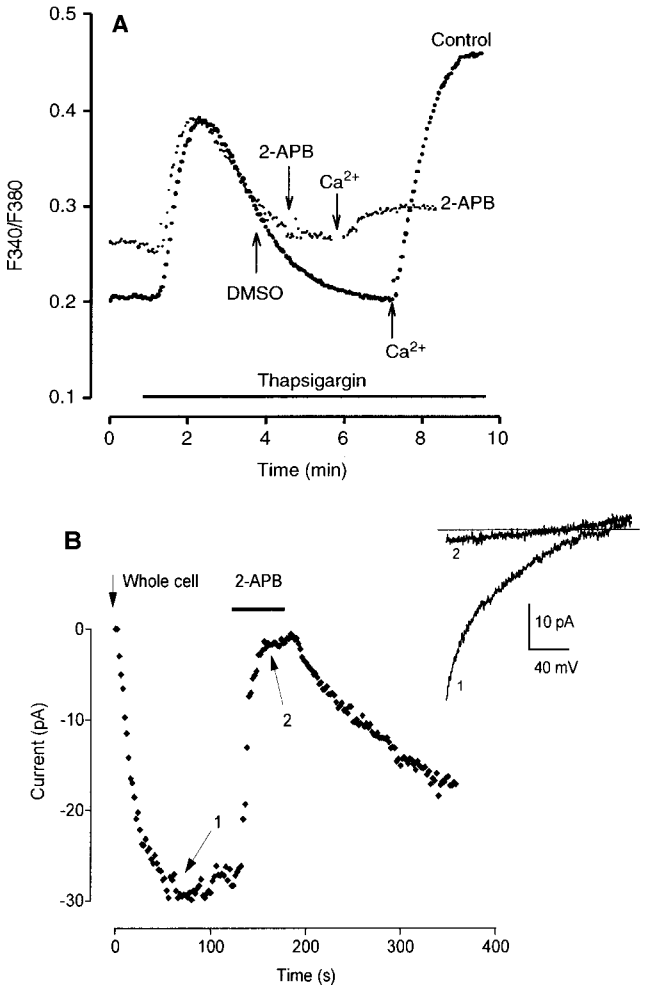

Figure 6 2-APB inhibits Ca^{2+} inflow to H4-IIE cells measured using fura-2 (A) or patch-clamp recording (B)

(**A**) H4-IIE cells loaded with fura-2 were incubated initially in the absence of added extracellular Ca^{2+}. The time of addition of thapsigargin (10 μM) is indicated by the horizontal bar and of 2-APB (100 μM), DMSO [0.1% (v/v), final concentration] and $CaCl_2$ (1.5 mM) by arrows. The traces shown are the means of the results obtained for 18–20 cells. (**B**) The time course for the activation of an inward current after the whole-cell configuration was achieved with 10 mM EGTA plus 20 μM Ins(1,4,5)P_3 in the pipette solution. 2-APB (50 μM) was added for the period indicated by the horizontal bar. Each point represents the amplitude of the current at -100 mV taken, at various times, from the current responses to voltage ramps applied every 2 s. The insert shows the instantaneous current–voltage relationship in the range -120 to $+60$ mV obtained in response to a voltage ramp corresponding to the times indicated in the plot of current as a function of time. The results shown are representative of those obtained for one of two experiments, each of which gave similar results.

DISCUSSION

The key observations reported here can be summarized as follows. (i) 2-APB inhibited thapsigargin- and vasopressin-stimulated Ca^{2+} inflow with half-maximal effects at about 10 μM. (ii) At concentrations of 2-APB which inhibited Ca^{2+} inflow, there was essentially no effect on the release of Ca^{2+} from intracellular stores induced by either vasopressin or thapsigargin. (iii) 2-APB had no effect on maitotoxin-stimulated Ca^{2+} inflow through non-selective cation channels [17–19]. (iv) The kinetics of the effects of 2-APB and Gd^{3+}, an inhibitor of Ca^{2+} inflow through SOCs [21], in inducing a decrease in $[Ca^{2+}]_{cyt}$ (following the addition of vasopressin and establishment of a plateau of $[Ca^{2+}]_{cyt}$) were indistinguishable. (v) 2-APB did not inhibit the Ins(1,4,5)P_3-induced release of Ca^{2+} from intracellular stores in permeabilized hepatocytes. (vi) 2-APB inhibited the Ins(1,4,5)P_3-induced inward current (equivalent to the Ca^{2+} release-activated Ca^{2+} current) in H4-IIE cells. (vii) The effects of 2-APB on Ca^{2+} inflow through SOCs were rapid in onset and were reversible.

The most likely explanation for these results is that 2-APB inhibits SOCs by directly interacting with the SOC pore, with another region of the SOC protein or with a closely associated regulatory protein. Another possible explanation is that 2-APB interacts with, and inhibits the function of, a small number of Ins(1,4,5)P_3 receptors involved in the activation of SOCs [i.e. that an Ins(1,4,5)P_3 receptor protein is a regulatory protein which is closely associated with the SOC protein (compare with [8,9])). However, this explanation is probably not the simplest explanation for the results presented here, and is considered unlikely, since no evidence was obtained to indicate that 2-APB inhibits any Ins(1,4,5)P_3-induced Ca^{2+} release from intracellular stores in liver cells. Moreover, 2-APB rapidly and reversibly inhibited the Ins(1,4,5)P_3-induced inward Ca^{2+} current in patch-clamp recording experiments after the current was established, suggesting that the effect of 2-APB is more likely to be directly on the SOC protein rather than indirectly on an Ins(1,4,5)P_3 receptor protein coupled to the SOC protein. Furthermore, there is no evidence to suggest that 2-APB selectivity inhibits a given subtype of Ins(1,4,5)P_3 receptor.

In contrast with the results obtained with HEK-293, pancreatic acinar and myometrial smooth muscle cells [8,9,11,22,23], no evidence was obtained to indicate that 2-APB inhibits Ins(1,4,5)P_3-induced Ca^{2+} release through Ins(1,4,5)P_3 receptors in liver cells {compared with the absence of an effect of 2-APB on agonist-receptor [Ins(1,4,5)P_3]-initiated Ca^{2+} release from intracellular

stores reported for cardiac myocytes [24], and for thapsigargin-initiated Ca^{2+} release in HEK-293 cells and smooth muscle cells [8,9]}. The absence of an effect of 2-APB on Ins(1,4,5)P_3-induced Ca^{2+} release is not due to the failure of 2-APB to diffuse across the liver cell plasma membrane and into the cytoplasmic space. Thus an increase in the time of preincubation of the cells with 2-APB did not affect the ability of vasopressin to release Ca^{2+}, and a high concentration of 2-APB was clearly effective in inducing the release of Ca^{2+} from intracellular stores in the absence of an agonist. Furthermore, 75 μM 2-APB did not inhibit Ins(1,4,5)P_3-induced Ca^{2+} release from intracellular stores in permeabilized cells, where it is expected that 2-APB would have ready access to the interior of the cells. The absence of inhibition by 2-APB of the release of Ca^{2+} from intracellular stores in liver cells, compared with the inhibition of this process observed in brain microsomes [10] and in HEK-293, pancreatic acinar and myometrial smooth muscle cells [8,9,11,22,23], may be due to differences in the subtypes of Ins(1,4,5)P_3 receptors expressed in the different cell types, the orientation and/or accessibility of Ins(1,4,5)P_3 receptors to 2-APB and/or to differences in the proportions of dimeric and monomeric species of 2-APB [9]. The observation that, in intact liver cells, 2-APB does not inhibit Ins(1,4,5)P_3 receptor function but does inhibit SOCs indicates that caution should be exercised in the use of 2-APB as a probe for the role of Ins(1,4,5)P_3 receptors in the activation of plasma membrane Ca^{2+} channels in studies of other animal cell types.

The observations that 2-APB inhibits thapsigargin- and vasopressin-stimulated Ca^{2+} inflow through SOCs, but not maitotoxin-stimulated Ca^{2+} inflow through non-selective cation channels, suggest that 2-APB may be a potentially useful inhibitor of SOCs. Such a reagent may assist in further studies of the mechanism of activation and physiological functions of SOCs in liver cells.

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