MK-886, a Leukotriene Biosynthesis Inhibitor, as an Activator of Ca$^{2+}$ Mobilization in Madin-Darby Canine Kidney (MDCK) Cells

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ABSTRACT

The effect of 3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-tert-butylthioindol-2-yl]-2, 2-dimethylpropanoic acid (MK-886), a leukotriene biosynthesis inhibitor, on Ca$^{2+}$ mobilization in Madin-Darby canine kidney cells has been examined by fluorimetry using fura-2 as a Ca$^{2+}$ indicator. MK-886 at 0.5 to 25 μM concentration dependently increased [Ca$^{2+}$]i. The [Ca$^{2+}$]i increase comprised an immediate initial rise and a slowly decaying phase. Ca$^{2+}$ removal inhibited the Ca$^{2+}$ signals by reducing both the initial rise and the decay phase, suggesting that MK-886 activated Ca$^{2+}$ influx and Ca$^{2+}$ release. In Ca$^{2+}$-free medium, 10 μM MK-886 still increased [Ca$^{2+}$]i, after pretreatment with carbonylcyanide m-chlorophenylhydrazone (CCCP; 2 μM), a mitochondrial uncoupler, and thapsigargin (1 μM), an endoplasmic reticulum Ca$^{2+}$ pump inhibitor. Conversely, pretreatment with MK-886 abolished CCCP- and thapsigargin-induced Ca$^{2+}$ release. This suggests that 10 μM MK-886 released Ca$^{2+}$ from the endoplasmic reticulum, mitochondria, and other stores. The addition of 3 mM Ca$^{2+}$ increased [Ca$^{2+}$]i after pretreatment with 10 μM MK-886 for 700 s in Ca$^{2+}$-free medium, indicating that MK-886 induced capacitative Ca$^{2+}$ entry. This capacitative Ca$^{2+}$ entry was partly inhibited by SKF96365 (50 μM), by econazole (25 μM), and by inhibiting phospholipase A$_2$ with arachidonic acid (40 μM) but not by inhibiting phospholipase D with 0.1 mM propranolol. MK-886 (10 μM)-induced Ca$^{2+}$ release was not altered by inhibiting phospholipase C with U73122 (2 μM) but was inhibited by 50% by suppressing phospholipase D and phospholipase A$_2$ with propranolol (0.1 mM) and arachidonic acid (40 μM), respectively.

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ABBREVIATIONS: MK-886, 3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-tert-butylthioindol-2-yl]-2, 2-dimethylpropanoic acid; DMEM, Dulbecco’s modified Eagle’s medium; ER, endoplasmic reticulum; fura-2/AM, 1-[2-(5-carboxyoxazol-2-yl)-6-aminozofuran-5-oxyl]-2-(2’-amino-5’-methylphenoxyo)-ethane-N,N,N,N-tetracetic acid pentaacetoxymethyl ester; IP$_3$, inositol 1,4,5-trisphosphate; MDCK, Madin-Darby canine kidney; U73122, 1-[6-[(17p)-3-methoxyestra-1,3,5(10)-tien-17-y]amino(ox)ethyl]-1H-pyrrole-2,5-dione; U73343, 1-[(6-[(17p)-3-methoxyestra-1,3,5(10)-tien-17-y]amino(ox)ethyl]-2,5-pyrrolidine-dione; CCCP, carbonylcyanide m-chlorophenyldihydrozone; SKF96365, 1-[[5-(4-methoxyphenyl)propoxy]4-methoxyphenethyl]-1H-imidazole hydrochloride.
cell events (Clapman, 1995; Berridge, 1997). A [Ca^{2+}], increase may occur on external stimulation as a result of Ca^{2+} entry and/or Ca^{2+} release. In nonexcitable cells that lack voltage-gated Ca^{2+} channels, one of the principle Ca^{2+} stores for the [Ca^{2+}], increase is the inositol 1,4,5-trisphosphate (IP_{3})-sensitive Ca^{2+} store (Berridge, 1993). Binding of IP_{3} to its receptors on the internal stores causes active release of internal Ca^{2+}. This discharge of the internal Ca^{2+} store often triggers Ca^{2+} influx, leading to a prolonged [Ca^{2+}], increase and refilling these stores. This Ca^{2+} influx is termed “capacitative Ca^{2+} entry” (Putney and Bird, 1993).

Here we have investigated the effect of MK-886 on Ca^{2+} signaling in Madin-Darby canine kidney (MDCK) cells. We have previously shown that in this epithelial cell, IP_{3}-dependent agonists such as ATP (Jan et al., 1998a) and bradykinin (Jan et al., 1999b) increase [Ca^{2+}], by releasing Ca^{2+} from the endoplasmic reticulum (ER) Ca^{2+} store, followed by capacitative Ca^{2+} entry. Additionally, thapsigargin (Jan et al., 1999a) and 2,5-di-tert-butylhydroquinone (Jan et al., 1999b) increase [Ca^{2+}], by inhibiting the ER Ca^{2+} pump without increasing IP_{3} levels, leading to Ca^{2+} release and capacitative Ca^{2+} entry. Thus, MDCK cells were chosen as a model to examine drug effects on Ca^{2+} homeostasis in nonexcitable cells.

Using fura-2 as a Ca^{2+} probe, we have found that MK-886 concentration dependently increased [Ca^{2+}], in MDCK cells. We have established the concentration-response relationships both in the presence and absence of external Ca^{2+} and have explored the possible mechanisms underlying MK-886-induced [Ca^{2+}], signals.

Materials and Methods

Cell Culture. MDCK cells obtained from American Type Culture Collection (CRL-6253; Manassas, VA) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO_{2}-containing humidified air.

Solutions. Ca^{2+} medium (pH 7.4) contained 140 mM NaCl, 5 mM KCl, 1 mM MgCl_{2}, 2 mM CaCl_{2}, 10 mM HEPES, and 5 mM glucose. Ca^{2+}-free medium contained no Ca^{2+} plus 1 mM EGTA (calculated [Ca^{2+}] < 0.1 nM). The experimental solution contained <1% of solvent (ethanol), which did not affect [Ca^{2+}], (n = 3).

Optical Measurements of [Ca^{2+}]. Trypsinized cells (10^6/ml) were loaded with 2 μM 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxyl]-2-[2’-amino-5’-methylphenoxyl]-ethane-N,N,N,N-tetraacetic acid pentaacetoxymethyl ester (fura-2/AM) for 30 min at 25°C in DMEM. Cells were washed and resuspended in Ca^{2+} medium before use. Fura-2 fluorescence measurements were performed in a water-jacketed cuvette (25°C) with continuous stirring; the cuvette contained 1 ml of medium and 0.5 million cells. Fluorescence was monitored with a Shimadzu RF-5301PC spectrofluorophotometer (Tokyo, Japan) by continuously recording excitation signals at 340 and 380 nm and emission signal at 510 nm at 1-s intervals.

Maximum and minimum fluorescence values were obtained by adding 0.1% Triton X-100 and 20 mM EGTA sequentially at the end of an experiment. [Ca^{2+}], was calculated as previously described (Gryniewicz et al., 1985). Our studies showed that trypsinized cells prepared by this protocol respond to stimulation with ATP (Jan et al., 1998a), bradykinin (Jan et al., 1999b) or thapsigargin (Jan et al., 1999a) similarly to cells attached to coverslips.

Chemical Reagents. The reagents for cell culture were from Life Technologies (Grand Island, NY). Fura-2/AM was from Molecular Probes (Eugene, OR). MK-886, 1-(6-(17β-3-methoxyestra-1,3,5(10)-trien-17β)-1H-pyrrole-2,5-dione (U73122), 1-(6-(17β-3-methoxyestra-1,3,5(10)-trien-17β)-1H-pyrrole-2,5-dione (U73122), 1-(6-(17β-

Results

MK-886 Induces [Ca^{2+}], Increases in MDCK Cells. At concentrations between 0.5 and 25 μM, MK-886 increased [Ca^{2+}], in Ca^{2+} medium (Fig. 1A). At a concentration of 0.1 μM, MK-886 had no effect (data not shown). Over a time period of 5 min, the [Ca^{2+}], increase consisted of an immediate initial rise and a slowly decaying phase. At a concentration of 10 μM, MK-886 induced a [Ca^{2+}], increase that reached a maximum height 180 s later at a net value of 502 ± 12 nM (Fig. 1A, trace b; n = 6; P < .05), followed by an elevated phase that had a net height of 351 ± 10 nM at the 350-s time point. The rise of the Ca^{2+} signal was slower in response to lower concentrations of MK-886. MK-886 at concentrations ≥50 μM caused a persistent increase in the fura-2 ratio signal, most likely reflecting cell membrane leakage; thus, these results were not reported.

Sources of MK-886-Induced [Ca^{2+}], Increases. Figure 1B shows that external Ca^{2+} removal decreased the Ca^{2+} signals induced by 1 to 25 μM MK-886, both in the maximum height and the area under the curve (30–350 s). The MK-886-induced increase in the fura-2 ratio signal was not a Ca^{2+}-insensitive artifact because, as shown in Fig. 1C, MK-886 (10 μM) induced an increase in the 340-nm excitation signal accompanied by a corresponding decrease in the 380-nm excitation signal. The concentration-response relationships of MK-886-induced [Ca^{2+}], increase both in the presence and absence of external Ca^{2+} are illustrated in Fig. 1D. The y-axis represents the net area under the curve of the [Ca^{2+}], increase.

The internal sources from which MK-886 mobilized Ca^{2+} were investigated. Figure 2A shows that in Ca^{2+}-free medium, 2 μM carbonylcyanide m-chlorophenylhydrazone (CCCP), a mitochondrial uncoupler, induced a small [Ca^{2+}], transient with a net peak height of 41 ± 5 nM (n = 6; P < .05), consistent with our previous reports (Jan et al., 1998b,c, 1999a,b,d). This [Ca^{2+}], increase was most likely caused by Ca^{2+} release from the mitochondria. Subsequently added thapsigargin (1 μM at 400 s), an ER Ca^{2+} pump inhibitor (Thastrup et al., 1990), lead to a marked [Ca^{2+}], increase with a net peak height of 118 ± 6 nM (n = 6; P < .05). Another ER Ca^{2+} pump inhibitor, cyclopiazonic acid (De Maurex et al., 1992), added at 800 s did not increase [Ca^{2+}],. This suggests that thapsigargin-sensitive ER Ca^{2+} stores had been completely depleted. However, 15 min after pre-treatment with CCCP, MK-886 (10 μM) still induced a [Ca^{2+}], increase with a net peak height of 52 ± 4 nM (n = 6; P < .05). Conversely, Fig. 2B shows that pretreatment with 10 μM MK-886 for 600 s in Ca^{2+}-free medium prevented thapsigargin (1 μM) and CCCP (2 μM) from releasing more Ca^{2+}.

Effects of MK-886 on Capacitative Ca^{2+} Entry. Because it was reported that depletion of internal Ca^{2+} stores often triggers capacitative Ca^{2+} entry in MDCK cells (Jan et al., 1998a,b,c, 1999a,b,c,d), experiments were performed to
examine whether MK-886-induced Ca\textsuperscript{2+} influx was via capacitative Ca\textsuperscript{2+} entry. Capacitative Ca\textsuperscript{2+} entry was measured by adding 3 mM Ca\textsuperscript{2+} to cells pretreated with MK-886 in Ca\textsuperscript{2+}-free medium. Figure 3A shows that after depleting the internal Ca\textsuperscript{2+} store for 880 s with 10 \(\mu\)M MK-886, Ca\textsuperscript{2+} induced an [Ca\textsuperscript{2+}] increase with a net maximum height of 487 ± 15 nM (trace a), which was higher than control (39 ± 5 nM; trace b) by 12-fold (\(n = 6; P < .05\)).

Figure 3A shows that adding SKF96365 (50 \(\mu\)M) and econazole (25 \(\mu\)M), two capacitative Ca\textsuperscript{2+} entry inhibitors (Jan et al., 1999c,d), at 750 s before addition of 3 mM CaCl\textsubscript{2} significantly inhibited MK-886-induced capacitative Ca\textsuperscript{2+} entry by 51 ± 5 and 49 ± 4\%, respectively, in the net area under the curve (900–1250 s).

Because we recently found that phospholipases A\textsubscript{2} and D may be involved in the regulation of Ca\textsuperscript{2+} signaling in MDCK cells (Jan et al., 1999e), the following experiments were performed to explore whether MK-886-induced capacitative Ca\textsuperscript{2+} entry is modulated by phospholipases A\textsubscript{2} and D. Figure 3B shows that after 10 \(\mu\)M MK-886 pretreatment for 700 s in Ca\textsuperscript{2+}-free medium, incubation with 40 \(\mu\)M aristolochic acid, a phospholipase A\textsubscript{2} inhibitor (Rosenthal et al., 1989), for 300 s before addition of 3 mM CaCl\textsubscript{2} significantly inhibited
MK-886-induced capacitative Ca\(^{2+}\) entry by 71 ± 5% in the net area under the curve (900–1150 s; \(n = 6\); \(P < .05\)). In contrast, substitution of arachidonic acid with 0.1 mM propranolol, a phospholipase D inhibitor (Billah et al., 1989), had no effect.

**Mechanism of MK-886-Induced Internal Ca\(^{2+}\) Release.** The pathway by which MK-886 releases Ca\(^{2+}\) was investigated by examining the effect of inhibiting phospholipase C-dependent IP\(_{3}\) formation. We have previously shown that ATP (10 \(\mu\)M) induces significant Ca\(^{2+}\) release in an IP\(_{3}\)-dependent manner (Jan et al., 1998c). Shown in Fig. 4A, trace a, is a typical [Ca\(^{2+}\)] increase induced by 10 \(\mu\)M ATP. Incubation with U73122 (1 \(\mu\)M), a phospholipase C inhibitor (Thompson et al., 1991), for 220 s abolished the [Ca\(^{2+}\)] increase induced by ATP (10 \(\mu\)M) (Fig. 4A, trace c; \(n = 6\); \(P < .05\)). This implies that U73122 pretreatment effectively blocked phospholipase C-dependent IP\(_{3}\) production. After U73122 pretreatment for 330 s, application of MK-886 (10 \(\mu\)M) induced a [Ca\(^{2+}\)] increase with a net maximum height of 140 ± 7 nM, which is 82 ± 5% (\(n = 6\); \(P < .05\)) of control (Fig. 4, trace b). U73343, an inactive U73122 analog, neither altered the resting [Ca\(^{2+}\)], nor the [Ca\(^{2+}\)] increases induced by ATP and MK-886 (data not shown). The effect of arachidonic acid and propranolol on MK-886-induced internal Ca\(^{2+}\) release was examined. Figure 4B shows that pretreatment with arachidonic acid (40 \(\mu\)M) for 300 s inhibited 10 \(\mu\)M MK-886-induced [Ca\(^{2+}\)], increase by 50 ± 6% in net peak height (trace c versus trace a; \(n = 6\); \(P < .05\)). Arachidonic acid did not alter the resting [Ca\(^{2+}\)]. Likewise, pretreatment with propranolol (0.1 mM) for 300 s markedly reduced 10 \(\mu\)M MK-886-induced [Ca\(^{2+}\)], peak by 51 ± 7% (Fig. 4B, trace b versus trace a; \(n = 6\); \(P < .05\)) without significantly increasing the resting [Ca\(^{2+}\)].

**Discussion**

This report is the first to demonstrate that MK-886, widely used as a 5-lipoxygenase inhibitor, induced a significant [Ca\(^{2+}\)] increase in a nonexcitable epithelial cell at concentrations commonly used to inhibit lipoxygenases. It is rather unlikely that the MK-886-induced increase in [Ca\(^{2+}\)], resulted from its inhibition of arachidonic acid metabolism because the other lipoxygenase inhibitors tested, such as baicalein (50 \(\mu\)M), 5,8,11,14-eicosatetraynoic acid (ETYA; 100–200 \(\mu\)M), caffeic acid (5–50 \(\mu\)M), esculetin (5–50 \(\mu\)M), and L-655238 (80–100 \(\mu\)M) did not alter the resting [Ca\(^{2+}\)], (data not shown). It is also unlikely that MK-886 increases [Ca\(^{2+}\)], by causing plasma membrane leakage because first, trypsin blue assay performed 20 min after cells were exposed to 25 \(\mu\)M MK-886 revealed no increased cell death than that in control; and second, Fig. 1A shows that the [Ca\(^{2+}\)] signals induced by 25 \(\mu\)M MK-886 reached a peak 3 min after drug application and started to decline afterward. If the increase in fura-2 ratio signal was due to Ca\(^{2+}\) influx through damaged plasma membrane, the fluorescence signal would increase persistently.

MK-886 triggers both Ca\(^{2+}\) influx and Ca\(^{2+}\) release at concentrations of 0.5 to 25 \(\mu\)M because the Ca\(^{2+}\) signals were partly decreased by Ca\(^{2+}\) removal. The rise and decay phases were both reduced by Ca\(^{2+}\) removal, suggesting that the [Ca\(^{2+}\)] increase involves Ca\(^{2+}\) influx throughout the whole course of measurement. Another line of evidence that MK-886 induces Ca\(^{2+}\) influx comes from Fig. 3 that illustrates that MK-886 (10 \(\mu\)M) induced capacitative Ca\(^{2+}\) entry.

The internal Ca\(^{2+}\) sources for MK-886-induced [Ca\(^{2+}\)] increase consist of thapsigargin-sensitive ER stores, CCCP-sensitive mitochondrial stores, and other unidentified stores. This is because in Ca\(^{2+}\)-free medium, pretreatment with 10 \(\mu\)M MK-886 prevented 1 \(\mu\)M thapsigargin and 2 \(\mu\)M CCCP from releasing more Ca\(^{2+}\); and conversely, after pretreating with CCCP and thapsigargin, MK-886 still released a significant amount of Ca\(^{2+}\). This is interesting because all the other Ca\(^{2+}\)-mobilizing substances we have tested so far in MDCK cells, such as ATP, bradykinin, U73122, cyclopiazonic acid, 2,5-di-tert-butylhydroquinone, econazole, and SKF96365,
release \(Ca^{2+}\) solely from thapsigargin-sensitive stores (Jan et al., 1998a,b,c, 1999a,b,c,d). Another possible candidate of internal \(Ca^{2+}\) stores is the ryanodine-sensitive store. However, it was previously shown that MDCK cells probably do not possess functional ryanodine receptors because neither ryanodine (1–50 \(\mu M\)) nor caffeine (10–20 \(mM\)) increases the resting \([Ca^{2+}]_i\) (Jan et al., 1998b). The unidentified \(Ca^{2+}\) stores were not further investigated due to the lack of selective pharmacological tools.

We have examined whether the \(Ca^{2+}\) release induced by MK-886 was mediated by a rise in cytosolic \(IP_3\) levels by using U73122, a phospholipase C inhibitor, to suppress \(IP_3\) formation. U73122 pretreatment resulted in a slight depletion of \(Ca^{2+}\) stores but completely blocked \(IP_3\) formation because ATP (10 \(\mu M\)) added subsequently did not increase \([Ca^{2+}]_i\). The lack of effect of ATP on \(Ca^{2+}\) release could not be due to U73122-induced partial depletion of \(Ca^{2+}\) stores because MK-886 added afterward still induced a \([Ca^{2+}]_i\) increase with a peak height only 15% smaller than that of control. The smaller \([Ca^{2+}]_i\) increase that MK-886 induced after U73122 pretreatment was most likely due to U73122-induced partial depletion of \(Ca^{2+}\) stores. Thus, it seems unlikely that \(IP_3\) has a dominant role in mediating MK-886-induced \(Ca^{2+}\) release.

It was shown in MDCK cells (Kennedy et al., 1997) that \(Ca^{2+}\)-mobilizing agents, such as bradykinin, can initiate a complex signaling cascade that includes early activation of upstream enzymes, including phospholipase C and phospholipase D, and phospholipase A_2-dependent release of arachidonic acid. Phospholipase A_2 is thought to move from cytosol to plasma membranes after an increase in \([Ca^{2+}]_i\). Interestingly, we found that MK-886-induced \(Ca^{2+}\) entry was inhibited by 50% by inhibiting phospholipase A_2 with aristolochic
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300 s reduced the MK-886 response by as much as 50% in peak height without significantly depleting Ca\(^{2+}\) stores.

We found that 10 μM MK-886 activates capacitative Ca\(^{2+}\) entry. This is consistent with the data shown in Fig. 1 that MK-886 activated significant Ca\(^{2+}\) influx. SKF96365 and econazole partly suppressed this Ca\(^{2+}\) entry, consistent with our previous results that these two drugs exerted partial inhibition of the capacitative Ca\(^{2+}\) entry induced by thapsigargin, cyclopiazonic acid, and UTP (Jan et al., 1999c,d).

Because another lipoxygenase inhibitor, nordihydroguaiaretic acid, has been shown to activate Ca\(^{2+}\)-dependent K\(^{+}\) channels in other cells (Hatton and Peers, 1996; Nagano et al., 1996), it is possible that the Ca\(^{2+}\) entry triggered by MK-886 in MDCK cells was caused by an increased driving force for Ca\(^{2+}\) influx that resulted from MK-886-induced membrane hyperpolarization by activating Ca\(^{2+}\)-dependent K\(^{+}\) channels. We examined this possibility by investigating the effect of valinomycin, a K\(^{+}\) ionophore, on [Ca\(^{2+}\)]\(_{i}\). Valinomycin was expected to hyperpolarize the cell by increasing K\(^{+}\) efflux. Our data suggest that during the 5 min of incubation with 10 to 100 μM valinomycin, the resting [Ca\(^{2+}\)]\(_{i}\) did not significantly increase (data not shown). Likewise, pretreatment with 10 to 20 mM tetraethylammonium and 10 μM charybdotoxin to inhibit K\(^{+}\) currents did not alter MK-886-induced [Ca\(^{2+}\)]\(_{i}\) increase. Thus, it appears that MK-886-induced [Ca\(^{2+}\)]\(_{i}\) increase is dissociated from its effects on membrane potential.

Figure 3A shows that in Ca\(^{2+}\)-free medium, the [Ca\(^{2+}\)]\(_{i}\) increase induced by 10 μM MK-886 remained elevated above prestimulatory baseline by approximately 50 nM, 700 s after drug addition. In contrast, it was shown that the [Ca\(^{2+}\)]\(_{i}\) increases induced by other ligands, such as ATP, bradykinin, thapsigargin, and 2,5-di-tert-butylhydroquinone (Jan et al., 1998a, 1999a,b), returned to baseline in less than 400 s after drug addition. One possible explanation is that MK-886 inhibits the mechanism underlying the efflux or sequestration of the mobilized Ca\(^{2+}\). We have found similar phenomena in econazole- and SKF96365-induced Ca\(^{2+}\) release (Jan et al., 1999c,d).

Collectively, we have characterized the [Ca\(^{2+}\)]\(_{i}\) increase induced by MK-886 in MDCK cells and have attempted to examine the possible underlying mechanisms. We have found several important effects of MK-886: 1) concentration dependently increasing [Ca\(^{2+}\)]\(_{i}\), at ranges commonly used to inhibit 5-lipoxygenase. [For example, in a study performed in myocytes, MK-886 was used at a concentration of 10 μM to demonstrate that this drug inhibited voltage-gated K\(^{+}\) currents while it activated Ca\(^{2+}\)-activated K\(^{+}\) currents (Smirnov et al., 1998)]; 2) activating Ca\(^{2+}\) influx and Ca\(^{2+}\) release; 3) releasing Ca\(^{2+}\) from thapsigargin-sensitive ER stores, CCCP-sensitive mitochondrial stores, and other stores; 4) triggering capacitative Ca\(^{2+}\) entry, which was inhibited by SKF96365, econazole, and aristolochic acid; and 5) mobilizing internal Ca\(^{2+}\) in an IP\(_3\)-independent, phospholipase D-, and phospholipase A\(_2\)-dependent manner. Given the fact that MK-886 increases [Ca\(^{2+}\)]\(_{i}\) in MDCK cells at concentrations commonly used by investigators to inhibit lipoxygenases in most cell types, we caution the use of this drug as a specific lipoxygenase inhibitor, especially in situations that increases in [Ca\(^{2+}\)]\(_{i}\), caused by Ca\(^{2+}\) influx and/or Ca\(^{2+}\) release may affect the results.

Fig. 4. A, trace a, ATP (10 μM) was added at 30 s. Trace b, control effect of MK-886 (10 μM; added at 330 s). Trace c, U73122 (2 μM) at 330 s. The experiments in A and B were both performed in Ca\(^{2+}\)-free medium. Traces are typical of five to six experiments.

acid but was not affected by suppressing phospholipase D with propranolol. This suggests that phospholipase A\(_2\)-coupled events, such as arachidonic acid synthesis, may have a positive feedback action both on MK-886-induced Ca\(^{2+}\) release and Ca\(^{2+}\) influx. In contrast, phospholipase D-associated events might be significantly involved in the modulation of MK-886-induced Ca\(^{2+}\) release but not Ca\(^{2+}\) influx. However, phospholipase D and phospholipase A\(_2\) may play a significant role in regulating MK-886-induced Ca\(^{2+}\) release because inhibition of phospholipase D and phospholipase A\(_2\) with propranolol (0.1 mM) and aristolochic acid (40 μM) for
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References


