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Kupffer Cells Contain Voltage-Dependent Calcium Channels

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SUMMARY

Kupffer cells, the resident hepatic macrophages, are activated by calcium, but conclusive evidence that they contain voltage-dependent calcium channels has not been presented previously. In this study, the cytosolic free calcium concentration ($[Ca^{2+}]_i$) of cultured Kupffer cells was measured with the fluorescent Ca^{2+} indicator fura-2. Partial replacement of extracellular Na^+ by K^+ caused an increase in $[Ca^{2+}]_i$ in a concentration-dependent manner (half-maximal effect at 81 mM K^+), presumably due to membrane depolarization. At 65 mM K^+, where there were minimal changes in $[Ca^{2+}]_i$, addition of the dihydropyridine-type calcium channel agonist BAY K 8644 (1 μM) caused a large increase in $[Ca^{2+}]_i$. Overall, the effect of BAY K 8644 (1 μM) was to shift the concentration-response curve for K^+ to the left (half-maximal effect at 61 mM K^+). Under depolarizing conditions (65 mM K^+), BAY K 8644 increased $[Ca^{2+}]_i$ in a concentration-dependent manner (half-maximal effect at approximately 400 nM BAY K 8644). Moreover, the dihydropyridine-type calcium channel blocker nitrendipine inhibited the BAY K 8644-induced increase in $[Ca^{2+}]_i$ in a concentration-dependent manner (half-maximal inhibition with about 25 nM nitrendipine). When extracellular Ca^{2+} was omitted from the incubation medium, the increases in $[Ca^{2+}]_i$ due to BAY K 8644 were prevented completely. In addition, an intracellular Ca^{2+} antagonist, 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate hydrochloride (200 μM), did not inhibit the BAY K 8644-sensitive, voltage-dependent increase in $[Ca^{2+}]_i$. Thus, these data collectively indicate that BAY K 8644 causes a transmembrane Ca^{2+} influx in Kupffer cells in a voltage-dependent manner, providing the first direct evidence that Kupffer cells contain L-type voltage-dependent Ca^{2+} channels.

Kupffer cells, which represent about 10% of all liver cells and constitute approximately 80% of the body's total fixed macrophages, clear foreign particles from the portal circulation and release biologically active compounds such as proteases, toxic radicals, eicosanoids, and cytokines (1). These cells have been implicated in liver injury and graft failure after liver transplantation (2) by producing inflammatory, cytotoxic, and vasoactive responses. Calcium is essential for activation of Kupffer cells (3, 4), and Ca^{2+} channel blockers increase graft survival after liver transplantation (5). The purpose of this study was to test the hypothesis that Kupffer cells contain L-type voltage-dependent Ca^{2+} channels.

The Ca^{2+} channel agonist BAY K 8644 prolongs opening of L-type voltage-dependent Ca^{2+} channels, promotes Ca^{2+} entry, and leads to stimulation of Ca^{2+}-dependent events in numerous cell types that posses L-type channels (6). Increases in $[Ca^{2+}]_i$ in response to membrane depolarization that is augmented by BAY K 8644 provide clear evidence for the presence of L-type Ca^{2+} channels. Therefore, in the present study we investigated the effect of BAY K 8644 on $[Ca^{2+}]_i$ in cultured Kupffer cells loaded with the Ca^{2+} indicator fura-2.

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Materials and Methods

Cell culture. Kupffer cells were cultured as described below. Livers from fed, female, Sprague-Dawley rats (220–280 g) were isolated under pentobarbital anesthesia (60 mg/kg of body weight, intraperitoneally) and perfused in a nonrecirculating system with Krebs-Ringer-HEPES buffer (115 mM NaCl, 5 mM KCl, 1 mM KH_2PO_4, 25 mM HEPES, 1 mM $CaCl_2$, pH 7.4, 37°), containing 0.016% collagenase (Boehringer Mannheim Biochemicals, Indianapolis, IN), for 10 min, after 10 min of perfusion with calcium-free Krebs-Ringer-HEPES buffer containing 0.5 mM EGTA (pH 7.4, 37°, saturated with 95% O_2/5% CO_2). Liver cells were dispersed by gentle shaking in phosphate-buffered saline (pH 7.4, 4°), and the nonparenchymal cell fraction was separated by centrifugation through Percoll gradients, essentially as described by Pertoft and Smedsrod (7). In order to purify Kupffer cells, nonparenchymal cells were resuspended in culture medium (RPMI 1640; GIBCO Laboratories Life Technologies Inc., Grand Island, NY) containing 10 mM HEPES, 100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B (2.0×10^6 cells/ml), seeded onto 25-

ABBREVIATIONS: $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; HEPES, [*N*-2-hydroxyethylpiperazide-*N*′-2-ethanesulfonic acid]; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; TMB-8, 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate hydrochloride; TNF, tumor necrosis factor.

mm glass coverslips, and cultured at 37° in a 5% CO_2 atmosphere (7). Nonadherent cells were removed 1 hr later by replacement with fresh culture medium. All flat cells on the coverslips phagocytosed 1-μm latex beads, verifying that they were Kupffer cells (8). Cultured Kupffer cells were used for this study within 16 hr of isolation.

Measurement of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was assessed fluorometrically, using the fluorescent calcium indicator fura-2 and a microspectrofluorometer. Kupffer cells were incubated in Krebs-Ringer-HEPES buffer, containing 5 μM fura-2/acetoxymethyl ester (Molecular Probes Inc., Eugene, OR), 0.06% Pluronic F127 (BASF Wyandotte, Wyandotte, MI), 1 mM $MgSO_4$, 5 mM glucose, and 2% bovine serum albumin, at 37° for 15 min and were allowed to equilibrate to 25° for 30 min. Coverslips plated with Kupffer cells were rinsed and placed in chambers with Krebs-Ringer-HEPES buffer, containing 1 mM $MgSO_4$ and 5 mM glucose, at 25°. Changes in fluorescence intensity of fura-2 at excitation wavelengths of 340 nm and 380 nm were monitored in individual Kupffer cells, using a Spex analytical system (Spex Industries Inc., Edison, NJ) interfaced with a NIKON Diaphot inverted microscope (9). Each value was corrected by subtracting the system dark-noise and autofluorescence, assessed by quenching fura-2 fluorescence with Mn^{2+}. $[Ca^{2+}]_i$ was determined as described by Grynkiewicz *et al.* (10) and Ratto *et al.* (11), from the equation:

$$[Ca^{2+}]_i = K_d \{(R-R_{min})/(R_{max}-R)\} (F_o/F_s)$$

where F_o/F_s is the ratio of fluorescent intensities evoked by 380-nm light from fura-2 pentapotassium salt in buffered salt solutions containing nanomolar Ca^{2+} ($[Ca^{2+}]_{min}$) and millimolar Ca^{2+} ($[Ca^{2+}]_{max}$) R is the ratio of fluorescent intensities at excitation wavelengths of 340 nm and 380 nm, R_{max} and R_{min} are values of R at $[Ca^{2+}]_{max}$ and $[Ca^{2+}]_{min}$, respectively. The values of these constants were determined at the end of each experiment. A K_d value of 135 nM was used (10).

In all experiments, Kupffer cells were incubated in Krebs-Ringer-HEPES buffer, and basal $[Ca^{2+}]_i$ was determined in the presence of 5 mM K^+. Buffer in the chamber was changed manually. Buffers containing high K^+ concentrations were prepared by substitution of an equimolar amount of NaCl with KCl. Solutions containing BAY K 8644 (Miles Inc., West Haven, CT), nitrendipine, and TMB-8 (Sigma Chemical Co., St. Louis, MO) were prepared by dilutions of 5, 30, and 400 mM stocks in ethanol, respectively. Ethanol itself has no effect on $[Ca^{2+}]_i$ at final concentrations used in this study.

Results

The increase of $[Ca^{2+}]_i$, in an individual representative Kupffer cell, caused by buffer containing 95 mM K^+ is illustrated in Fig. 1. For the first 2 min, $[Ca^{2+}]_i$ increased slowly from basal $[Ca^{2+}]_i$ values of <100 nM and then increased rapidly to peak values around 500 nM. A subsequent rapid decline to new steady state values of approximately 200 nM was observed in the continued presence of 95 mM K^+; however, when the external solution was replaced with buffer containing 5 mM K^+, $[Ca^{2+}]_i$ returned rapidly to basal values.

The concentration-response curve for extracellular K^+ is shown in Fig. 2. In the absence of BAY K 8644, extracellular K^+ increased $[Ca^{2+}]_i$ in a concentration-dependent manner in the concentration range between 75 mM and 95 mM (half-maximal effect at 81 ± 1 mM K^+). The increase in $[Ca^{2+}]_i$ observed in buffer containing less than 75 mM K^+ was minimal, whereas the maximal increase occurred with about 90 mM K^+. In the presence of BAY K 8644 (1 μM), the $[Ca^{2+}]_i$ response curve was shifted to the left. Under these conditions, the half-maximal effect was observed with 61 ± 1 mM K^+ ($p < 0.001$ for comparison with value in the absence of BAY K 8644; Student's t test).

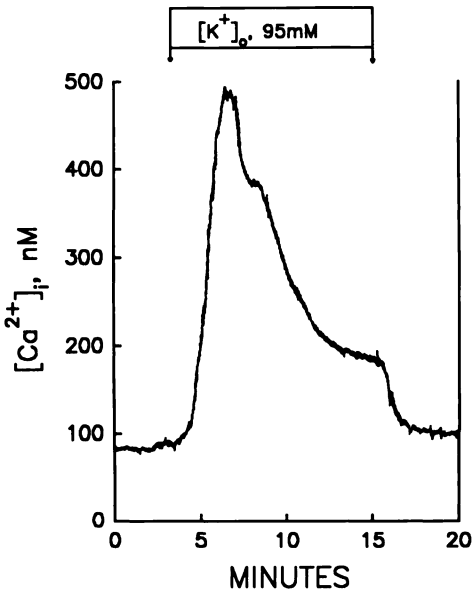

Fig. 1. Effect of depolarization on intracellular Ca^{2+} in an isolated Kupffer cell. The $[Ca^{2+}]_i$ in an isolated Kupffer cell was assessed fluorometrically, using the fluorescent calcium indicator fura-2, as described in Materials and Methods. KCl in Krebs-Ringer-HEPES buffer was increased to 95 mM (see Materials and Methods) during the time indicated by the *arrows* and *horizontal bar*. $[K^+]_o$, extracellular potassium concentration. The data are representative of experiments repeated four times.

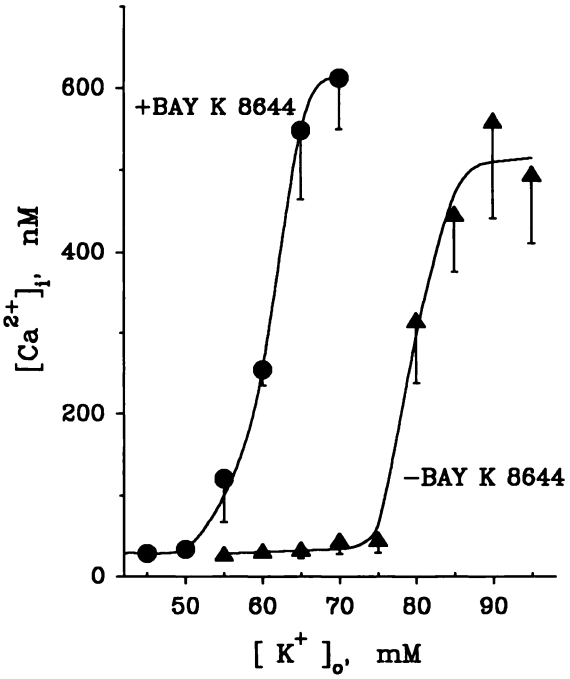

Fig. 2. Effect of extracellular K^+ concentration on intracellular Ca^{2+} in isolated Kupffer cells in the presence and absence of BAY K 8644. Experimental conditions were as described in Fig. 1. The extracellular potassium concentration ($[K^+]_o$) was increased from 5 mM to concentrations indicated on the *abscissa*, in the presence (●) and absence (▲) of BAY K 8644 (1 μM). $[Ca^{2+}]_i$ was measured at the peak of the response. Data are expressed as the mean ± standard error of four individual experiments per group.

As suggested by the results shown in Fig. 2, when cells were incubated in buffer containing 65 mM K^+ and no BAY K 8644, $[Ca^{2+}]_i$ tended to increase (Fig. 3), but the effect was not statistically significant (Fig. 2; Table 1). Similarly, under basal

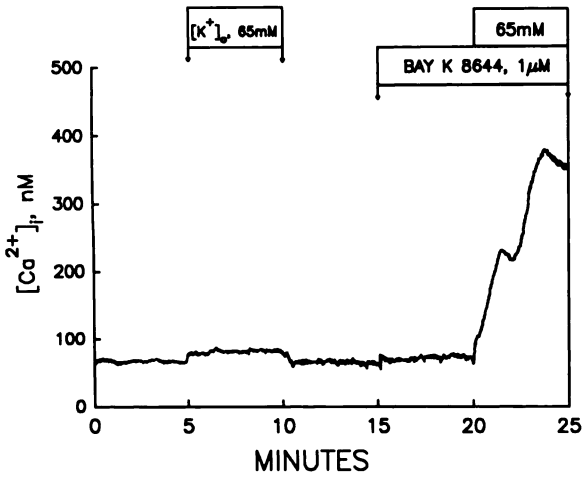

Fig. 3. Effect of BAY K 8644 and elevated K^+ on intracellular Ca^{2+} in an isolated Kupffer cell. Experimental conditions were as described in Fig. 1. Addition of BAY K 8644 (1 μM) and elevated K^+ are indicated by *arrows* and *horizontal bars*. $[K^+]_o$, extracellular potassium concentration. This is a typical experiment, which was repeated four times.

TABLE 1

Effect of BAY K 8644 on cytosolic free calcium in isolated Kupffer cells

Experimental procedures were as described in the legend to Fig. 3. $[K^+]_o$, extracellular potassium concentration. Data are expressed as the mean ± standard error of four individual measurements.

$[K^+]_o$	BAY K 8644	$[Ca^{2+}]_i$
mM	μM	nM
5	0	86 ± 9 (basal)
65	0	94 ± 7
5	1	104 ± 16
65	1	602 ± 91[a]

[a] $p < 0.01$ for comparison with all other values (paired Student's *t* test).

conditions (5 mM K^+), BAY K 8644 had no effect on $[Ca^{2+}]_i$ (Fig. 3). In contrast, in the presence of 1 μM BAY K 8644, 65 mM K^+ increased $[Ca^{2+}]_i$ markedly in the same cells (Fig. 3; Table 1). Because partial substitution of Na^+ with K^+ alone did not increase $[Ca^{2+}]_i$, it is unlikely that the Na^+/Ca^{2+} exchanger is responsible for the BAY K 8644-sensitive increase in $[Ca^{2+}]_i$.

The effect of different BAY K 8644 concentrations on $[Ca^{2+}]_i$ is shown in Fig. 4. BAY K 8644 increased $[Ca^{2+}]_i$ in a concentration-dependent manner, with near-maximal increases observed at values around 1 μM. The concentration of BAY K 8644 needed to cause half-maximal increase was around 400 nM. Because the effect of 1 μM BAY K 8644 on $[Ca^{2+}]_i$ was maximal at 65 mM K^+ (Fig. 2), subsequent experiments were performed with 1 μM BAY K 8644 in depolarizing buffer containing 65 mM K^+.

To determine whether the increase in $[Ca^{2+}]_i$ caused by membrane depolarization in 65 mM K^+ in the presence of BAY K 8644 could be inhibited by Ca^{2+} channel blockers, the effects of nitrendipine, a dihydropyridine-type Ca^{2+} channel blocker, was tested. The concentration-response curve of nitrendipine for the BAY K 8644-induced increase in $[Ca^{2+}]_i$ is depicted in Fig. 5. In the presence of nitrendipine at concentrations equal to or above 30 nM, the increase in $[Ca^{2+}]_i$ was reduced markedly, and half-maximal inhibition was achieved at concentrations of nitrendipine around 25 nM.

If L-type Ca^{2+} channels were required for the increase in

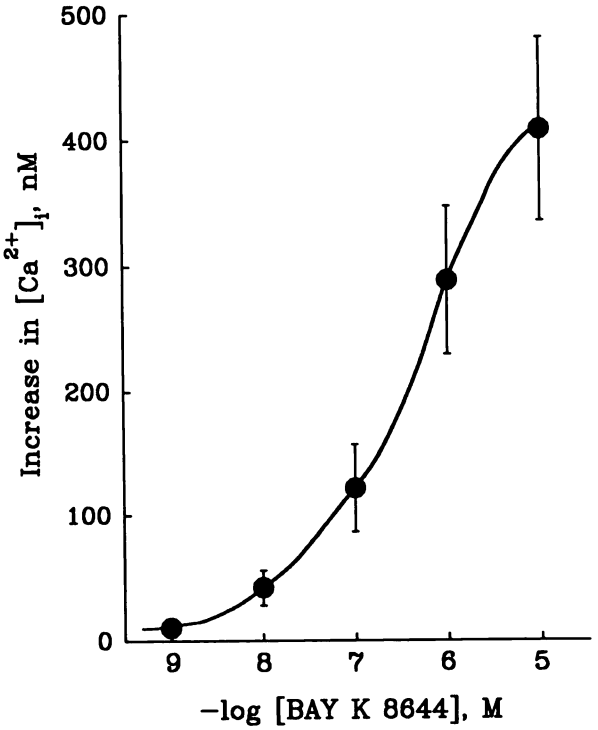

Fig. 4. Effect of BAY K 8644 concentration on intracellular Ca^{2+} in isolated Kupffer cells. Experimental conditions were as described in Fig. 1. The concentration of BAY K 8644 was increased in a stepwise manner, in buffer containing 65 mM K^+. $[Ca^{2+}]_i$ was measured at the peak of the response. Data are expressed as the mean ± standard error of four individual experiments.

$[Ca^{2+}]_i$, the effect should depend on the presence of extracellular calcium. Therefore, the dependence of the BAY K 8644-induced $[Ca^{2+}]_i$ increase on extracellular Ca^{2+} was also examined (Fig. 6; Table 2). In the presence of 1 mM extracellular Ca^{2+}, $[Ca^{2+}]_i$ increased significantly when the Ca^{2+} channel agonist BAY K 8644 (1 μM) was added under depolarizing conditions (Figs. 3 and 6). However, when the external solution was replaced with Ca^{2+}-free Krebs-Ringer-HEPES buffer containing 1 mM EGTA, $[Ca^{2+}]_i$ decreased immediately to basal values. Then, in the absence of extracellular Ca^{2+}, a second addition of BAY K 8644 had no effect. In contrast, when extracellular Ca^{2+} was restored in the same cells in the presence of BAY K 8644, $[Ca^{2+}]_i$ increased again dramatically, to values around 400 nM (Fig. 6; Table 2).

To test whether the release of Ca^{2+} from intracellular Ca^{2+} pools is involved in the BAY K 8644-induced increase in $[Ca^{2+}]_i$, an agent that blocks release of Ca^{2+} from intracellular stores, TMB-8, was studied. BAY K 8644 increased $[Ca^{2+}]_i$ to values of 426 ± 64 nM in response to depolarization. Addition of BAY K 8644 (1 μM) in depolarizing buffer after 10 min of preincubation with TMB-8 (200 μM) increased $[Ca^{2+}]_i$ to 460 ± 28 nM, values similar to those observed in the absence of TMB-8. Thus, intracellular stores do not appear to contribute to the BAY K 8644-induced elevation of intracellular Ca^{2+} in Kupffer cells observed in this study, at least in experiments with 200 μM TMB-8.

Discussion

L-type voltage-dependent calcium channels are known to exist in both excitable and nonexcitable tissues (12). Calcium

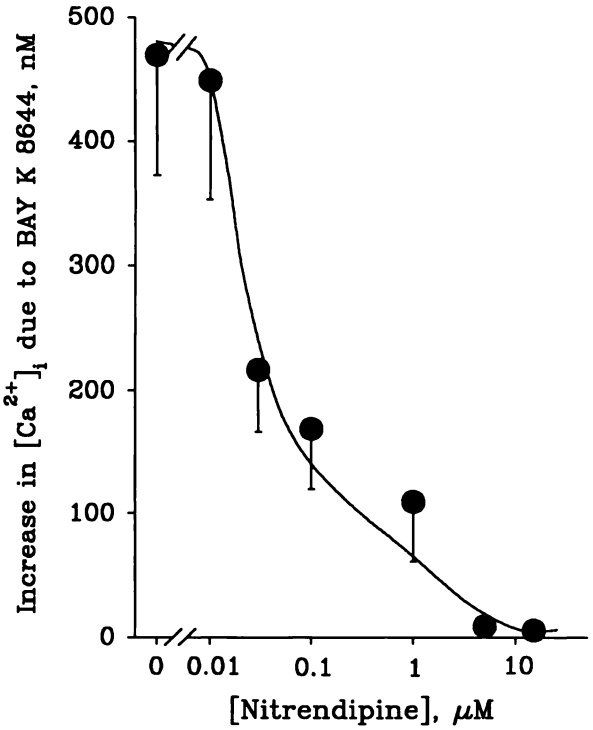

Fig. 5. Effect of nitrendipine concentration on BAY K 8644-induced increase in intracellular Ca^{2+} in isolated Kupffer cells. Experimental conditions were as described in Fig. 1. Isolated Kupffer cells were preincubated with nitrendipine, in buffer containing 5 mM K^+, for 8 min before addition of BAY K 8644 (1 μM), in buffer containing 65 mM K^+. The peak values of $[Ca^{2+}]_i$ were plotted. Data are expressed as the mean ± standard error of four individual measurements.

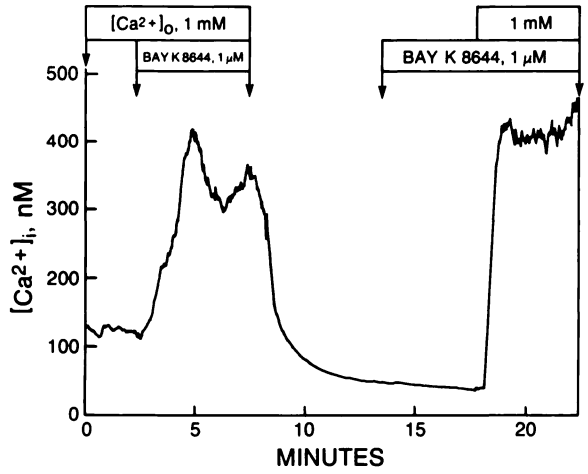

Fig. 6. Dependence of BAY K 8644-induced increase in intracellular Ca^{2+}, in an isolated Kupffer cell, on extracellular Ca^{2+}. Experimental conditions were as described in Fig. 1. Addition of BAY K 8644 (1 μM) and Ca^{2+} (1 mM) in Krebs-Ringer-HEPES buffer is indicated by *arrows* and *horizontal bars*. The potassium concentration was increased from 5 to 65 mM at 2 min and kept at 65 mM throughout the experiment (i.e., until 22 min). Kupffer cells were incubated with Ca^{2+}-free Krebs-Ringer-HEPES buffer containing 1 mM EGTA from 7 to 17 min. *$[Ca^{2+}]_o$*, extracellular calcium concentration.

channel blockers, such as nifedipine and verapamil, inhibit calcium influx across cell membranes and are used clinically in the treatment of several cardiovascular diseases (12). Lymphocytes have also been reported to possess voltage-dependent Ca^{2+} channels (13). Calcium channel blockers decrease TNF production (14), tumoricidal activity (15), and synthesis of the

TABLE 2

Effect of extracellular Ca^{2+} on BAY K 8644-induced increase in intracellular Ca^{2+} in isolated Kupffer cells

Experimental procedures were as described in the legend to Fig. 6. $[Ca^{2+}]_o$, extracellular calcium concentration; $[K^+]_o$, extracellular potassium concentration. Data are expressed as the mean ± standard error of nine individual measurements.

$[Ca^{2+}]_o$	$[K^+]_o$	BAY K 8644	$[Ca^{2+}]_i$
mM	mM	μM	nM
1	5	0	78 ± 11 (basal)
1	65	1	200 ± 54*
0	65	0	38 ± 4
0	65	1	35 ± 4
1	65	1	351 ± 77*

* $p < 0.05$ for comparison with basal value (paired Student's *t* test).

complement factor C_2 (16) by monocytes and resident macrophages, which originally differentiate from stem cells in bone marrow, where lymphocytes also originate. Moreover, the calcium channel agonist BAY K 8644 stimulates C_2 synthesis by monocytes (16). Thus, calcium channel blockers modulate monocytes and macrophages in several tissues. This study was designed to test the hypothesis that Kupffer cells, the resident hepatic macrophages, contain L-type voltage-dependent calcium channels, by directly measuring the changes in $[Ca^{2+}]_i$ in response to membrane depolarization and agents known to affect L-type Ca^{2+} channels in other cell types. Based on the facts that 1) the increase in $[Ca^{2+}]_i$ observed in Kupffer cells could be provoked by depolarization, 2) the increase in $[Ca^{2+}]_i$ was stimulated by a dihydropyridine-type Ca^{2+} channel agonist (BAY K 8644) and inhibited by an antagonist (nitrendipine), and 3) the increase was dependent on extracellular Ca^{2+}, we conclude that Kupffer cells contain L-type voltage-dependent calcium channels.

Voltage-dependent $[Ca^{2+}]_i$ increase in Kupffer cells. The increase in extracellular K^+ concentration depolarizes the plasma membrane and increases $[Ca^{2+}]_i$ in cells containing voltage-dependent Ca^{2+} channels (17, 18). In Kupffer cells, elevated K^+ increased $[Ca^{2+}]_i$ in a concentration-dependent manner, indicating that the increase in $[Ca^{2+}]_i$ was voltage dependent (Fig. 2). Depolarization with 95 mM K^+ caused a marked increase in $[Ca^{2+}]_i$, followed by a rapid decline to new steady state values (Fig. 1). A decline in $[Ca^{2+}]_i$ after a peak has been reported in neurons (19) and is most likely due to time-dependent inactivation of calcium channels, as well as to cellular Ca^{2+} extrusion and sequestration mechanisms.

In neurons, 10 mM K^+ itself causes a small increase in $[Ca^{2+}]_i$ (19), and half-maximal increases in $^{45}Ca^{2+}$ influx in synaptosomes were obtained at about 35 mM external K^+ (17). Similarly, Abe *et al.* (18) reported that half-maximal increases in $[Ca^{2+}]_i$ were observed at 25.5 mM K^+ in the coronary artery of the pig. In contrast, $[Ca^{2+}]_i$ did not increase with K^+ concentrations less than 75 mM in Kupffer cells (half-maximal concentration, 81 mM). The reason why isolated Kupffer cells require larger voltage steps to increase $[Ca^{2+}]_i$ than do excitable cells is unclear. One possibility is that Kupffer cells may have a low density of calcium channels (see below). Because the amount of Ca^{2+} influx depends on the total number of open Ca^{2+} channels, in Kupffer cells (with relatively few Ca^{2+} channels) it may be necessary to open more channels before $[Ca^{2+}]_i$ increases are observed. Because L-type channels are voltage dependent, a higher open probability would be achieved at greater K^+ concentrations. Alternatively, Kupffer cells may contain only L-type voltage-dependent Ca^{2+} channels, whereas

neurons and muscle cells contain T-type channels, which are activated at more negative membrane potentials than L-type channels (20). Additionally, Percoll is taken up by phagocytosis and has been reported to affect some cell surface-related phenomena in Kupffer cells (21). Thus, it might have modified the voltage dependency of isolated Kupffer cells.

Sensitivity to dihydropyridines. Multiple types of voltage-dependent calcium channels, such as L-, T-, or N-type, have been reported (22), and only L-type calcium channels are sensitive to dihydropyridines (20). The dihydropyridine-type calcium channel agonist BAY K 8644 prolongs opening of L-type channels (6, 23) and shifts the voltage dependence of open probability to more negative potentials (20). In Kupffer cells, BAY K 8644 shifted the concentration-response curve for K^+ to the left (Fig. 2). Further, the increase in $[Ca^{2+}]_i$ caused by BAY K 8644 was concentration dependent (Fig. 4) and was blocked by the dihydropyridine-type calcium channel blocker nitrendipine (Fig. 5). These findings indicate that the voltage-dependent increase in $[Ca^{2+}]_i$ was due to Ca^{2+} influx via L-type voltage-dependent Ca^{2+} channels.

The potency of dihydropyridines in blocking L-type channels is very different in various tissues; dihydropyridines are much more potent on smooth muscle L-type channels than on neuronal channels, suggesting that a heterogeneity among L-type channels exists (24). In guinea pig myocytes and frog ventricular cells, half-maximal effects of BAY K 8644 on calcium channel currents carried by Ba^{2+} were observed at concentrations between 30 and 100 nM (23). In a pituitary cell line, half-maximal concentrations of BAY K 8644 for prolactin secretion were approximately 500 nM (25). The half-maximal concentration of 400 nM observed in Kupffer cells in this study (Fig. 4) is within the range of values observed among different tissues studied to date.

The dihydropyridine-type Ca^{2+} channel blockers are known to bind to the α_1 subunit of L-type voltage-dependent Ca^{2+} channels and many other cellular proteins, such as the nucleoside transporter and the glycoprotein involved in multidrug resistance (26). The L-type Ca^{2+} channel has high affinity for antagonists, with K_d values in the nanomolar range, whereas the other sites have low affinity and high capacity (26). We have reported previously that nitrendipine binds specifically to Kupffer cells (27). In this study, we observed specific binding to [^{3}H]PN 200–110, another dihydropyridine-type Ca^{2+} channel antagonist, to isolated Kupffer cells. A high affinity binding site, with K_d and B_{max} values estimated to be around 0.4 nM and 1 fmol/10^6 cells, respectively, was observed. However, Kupffer cells also contained other binding sites with much lower affinity (K_d > 100 nM) and higher capacity (27), making characterization of the high affinity sites difficult.

Source of Ca^{2+} responsible for increase in $[Ca^{2+}]_i$ due to BAY K 8644. $[Ca^{2+}]_i$ can be increased via transmembrane influx of extracellular Ca^{2+} and/or release of Ca^{2+} from intracellular pools. BAY K 8644 increased $[Ca^{2+}]_i$ only in the presence of extracellular Ca^{2+} (Fig. 6; Table 2), indicating that the BAY K 8644-sensitive, voltage-dependent increase in $[Ca^{2+}]_i$ was due to transmembrane Ca^{2+} influx into the cell. The intracellular Ca^{2+} antagonist TMB-8 inhibits Ca^{2+} release from intracellular Ca^{2+} stores (28). For example, TMB-8 (5 μM) inhibits the caffeine-induced release of $^{45}Ca^{2+}$ from the sarcoplasmic reticulum preparation of skeletal muscle (28), and histamine release from rat mast cells is also diminished 74% with 200 μM TMB-8 (29). In Kupffer cells, however, 200 μM TMB-8 did not affect the increase in $[Ca^{2+}]_i$ caused by BAY K 8644, suggesting that intracellular Ca^{2+} pools are not involved in the dihydropyridine-sensitive, voltage-dependent increase in $[Ca^{2+}]_i$.

Clinical relevance of L-type Ca^{2+} channels in Kupffer cells. Based on the results presented here showing that Kupffer cells contain L-type Ca^{2+} channels, we predict that Ca^{2+} channel blockers would prevent damage to liver cells. In support of this idea, Takei *et al.* (5) showed that the L-type Ca^{2+} channel blocker nisoldipine improved graft survival after cold storage and transplantation of rat liver, actions that were confirmed in isolated perfused rabbit liver (30). We recently found that nisoldipine decreased TNF release from liver after transplantation in this model (31). Patients with alcoholic hepatitis or alcoholic cirrhosis frequently exhibit endotoxemia, leading to the production of TNF by Kupffer cells (32, 33). Therefore, modulation of Kupffer cell function by blocking of Ca^{2+} channels represents a new approach to treatment of hepatic pathological conditions such as alcoholic liver injury and improvement in procedures such as liver transplantation.

Acknowledgments

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