EFFECTS OF THE CALCIUM CHANNEL AGONIST, BAY K 8644, ON ELECTRICAL ACTIVITY IN MOUSE PANCREATIC B-CELLS

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ABSTRACT: We studied the effects of the dihydropyridine derivative BAY K 8644 on the membrane potential of B-cells in mouse pancreatic islets. BAY K 8644, in a dose-dependent manner, decreased the spike frequency but increased the duration of the spikes elicited by glucose with or without quinine or tetraethylammonium (TEA). These effects were antagonized by cobalt and nifedipine but not by tetrodotoxin. The interval between spikes was proportionate to the duration of the spikes and the ratio of the interval to the spike duration was constant at all concentrations of BAY K 8644 tested. Peak inward current, estimated from the derivative of the action potential recorded in the presence of TEA, was increased by BAY K 8644 and decreased by nifedipine. BAY K 8644 elicited spike activity when the membrane was moderately depolarized by either 5.6 mM glucose or 15 mM K^+, but did not change the membrane potential of the resting hyperpolarized B-cell. These results suggest that BAY K 8644 acts on the open Ca^{2+}-channels. The threshold occurs at a membrane potential of -50 mV. Also, the modifications of the shape of the spikes appear to reflect specific changes in Ca^{2+} entry. We propose the existence of a Ca^{2+}-induced Ca^{2+}-channel inactivation process in the pancreatic B-cell.

INTRODUCTION

Calcium entry is known to represent a critical event in the stimulus-secretion coupling process of a wide variety of secretory cells (26). In pancreatic B-cells, there is a large body of evidence emphasizing the role of Ca^{2+} in the process of glucose-induced insulin release (28). The recognition of glucose by the pancreatic B-cell involves a membrane channel modulation that in turn triggers the characteristic B-cell electrical activity (4, 20). Numerous studies performed to elucidate the ionic mechanisms underlying the glucose-induced electrical activity point out the role of modifications in both the Ca^{2+} and K^+ conductances (3–5, 21, 24, 25).

Different Ca^{2+}-channel blockers, such as divalent cations (Co^{2+}, Mn^{2+}, Mg^{2+}) dihydropyridines (Nifedipine, nitrendipine), and phenylalkylamines (Verapamil, D 600) have been used to study Ca^{2+} channel properties in many cell types, including the pancreatic B-cell (2, 11, 12, 14, 21, 25). Recently, it has been shown that structural modifications of dihydropyridine molecules generate a novel class of components (BAY K 8644, CGP 28392), which have been proposed to act as Ca^{2+}-channel agonists instead of antagonists. These agonists have already been shown to stimulate cardiac and vascular smooth muscle (23, 27) and to enhance stimulated catecholamine (10), aldosterone (15), prolactin (9), and insulin secretion (19).

The aims of the present study were to characterize the effects of the new "Ca agonist," BAY K 8644, on pancreatic B-cells whose electrical activity exhibits a strong Ca^{2+} dependency in order to gain further insight into the Ca^{2+}-channel properties of these cells in intact islets.

MATERIALS AND METHODS

All experiments were performed with islets of Langerhans microdissected from the tail portion of pancreases removed from 3-mo-old female Swiss Webster albino mice. A single islet of Langerhans was mounted in a perifusion chamber (40 μl volume) and was continuously perifused with a modified Krebs' solution (120 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 2.56 mM $CaCl_2$, 1.1 mM $MgCl_2$), equilibrated with a mixture of O_2 (95%) and CO_2 (5%). The flow rate was adjusted to 1.5 ml/min and the temperature was maintained at 37°C. The medium contained, as required: glucose, quinine hydrochloride, tetraethylammonium chloride (TEA), and tetrodotoxin (TTX) (Sigma Chemical Co., St. Louis, MO); cobalt chloride ($CoCl_2$) (Fisher Scientific Co. Silver Springs, MD); BAY K 8644 (Miles Laboratories, New Haven, CT); and Nifedipine (Pfizer, Inc., Brooklyn, NY). When TEA or high concentrations of KCl were added to the medium, the concentration of NaCl was lowered accordingly to keep osmolarity constant.

Both BAY K 8644 and Nifedipine were first dissolved in dimethylsulfoxide (DMSO) and further diluted in the perifusing medium so that the final concentration of the organic solvent never exceeded 0.1% (vol/vol). The control medium contained the same concentration of DMSO as the experimental medium. DMSO up to 0.1% failed to affect the B-cell membrane potential or electrical activity. Experiments were conducted with minimal light to prevent photodegradation of the dihydropyridines.

The electrophysiological methods used in the present study have been described in detail elsewhere (1). Briefly, the membrane potential was

measured between two Ag-AgCl electrodes, one in the bathing solution and the other in the intracellular micro-electrode. The microelectrodes were prepared with 0.7 mm ID single glass capillary tubing with an inner fiber (Frederick Haer, Brunswick, MN) and pulled with a Narishige model PE 2 electrode puller. The glass microelectrodes were filled with a 1:1 mixture of 3 M KCl and 2 M KCitrate and had tip resistances of $\sim 2 \times 10^8$ Ω. Membrane potential was recorded using a low noise differential amplifier. To identify the B-cell, we made impalements in the presence of 11.1 mM glucose. Experiments were performed on cells that showed a stable membrane potential and a regular burst pattern of activity during a 15-min period. Membrane potential was recorded on a 4-channel magnetic tape recorder (Store 4, Racal Thermionic Ltd., Sarasota, FL) and reproduced on a dual-channel ink recorder (Brush recorder, model 220 [Gould, Inc., Oxnard, CA]).

For small cells, the cytosolic potential may be considered to be equi-potential and the ionic current flowing through the membrane (I_i) can be taken to be proportional to the rate of depolarization (dV/dt) (7). Thus, $I_i = -C_m\, dV/dt$, where C_m represents the membrane capacity and dV/dt the time derivative of the membrane potential. Assuming a pancreatic B-cell surface area of 500 μm^2 and a membrane capacity of 1 $\mu F/cm^2$, the analysis of the maximum rate of depolarization, $dV/dt(max)$, of the spike can give a good approximation of the peak inward current.

The maximum rate of depolarization of the action potential was measured using a Nicolet 4094 digital oscilloscope (Nicolet Scientific Corp., Northvale, NJ). The duration of the spikes was calculated as the time spent between the ascending phase and the descending phase, taking the foot of the spike as the reference membrane potential. The interval between spikes was taken as the time between the bottom of the descending phase and the foot of the following spike. Each test substance was added for at least 5 min and the measurements of steady-state spike frequency, intervals between spikes, and spike duration were made during the last 3 min of exposure to the drug. During this study, 62 experiments were performed and the figure are representative of typical experiments.

RESULTS

Effects of BAY K 8644 at Different Membrane Potentials

In the absence of glucose, the pancreatic B-cell membrane potential remains at a hyperpolarized level around −70 mV (Fig. 1 *A*). Under these conditions, the addition of BAY K 8644 (1 μM) did not induce measurable changes in the resting membrane potential.

Increasing the glucose concentration to 5.6 mM depolarized the membrane, which reached a new stable potential around −50 mV (Fig. 1 *B*). In the presence of 5.6 mM glucose, a 40-mV amplitude spike was recorded 90 s after the addition of BAY K 8644 (1 μM). The spike was followed by a long lasting and slowly reversible hyperpolarization; and when the membrane potential reached its previous level, a new spike was elicited. The second spike was recorded after the removal of BAY K 8644. In two experiments, when the depolarizing effect of 5.6 mM

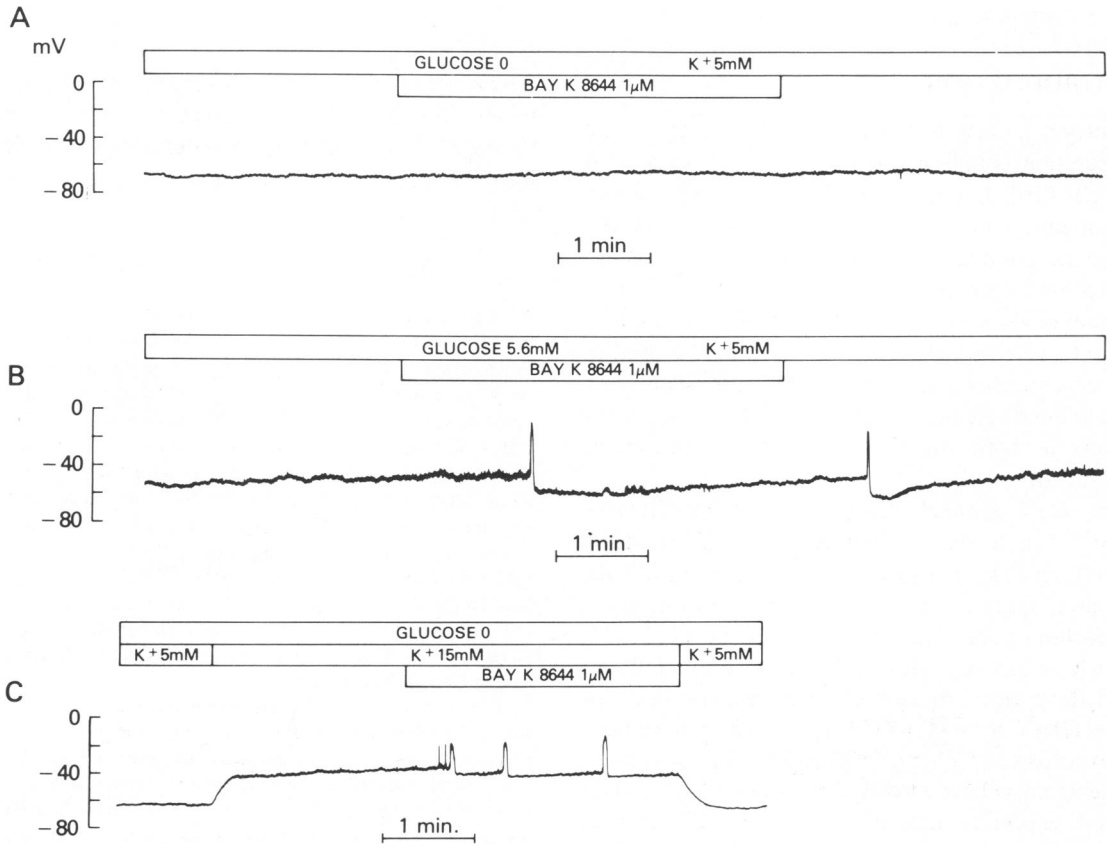

FIGURE 1 Effects of BAY K 8644 at different membrane potentials. Effects of 1 μM BAY K 8644 on B-cell membrane potential recorded in the absence of glucose (*A*), in the presence of 5.6 mM glucose (*B*), or in the presence of 15 mM K^+ (*C*) in the external medium.

glucose was <15 mV, the addition of BAY K 8644 (1 μM) failed to induce the appearance of spikes.

In the absence of glucose, a sudden elevation of external K^+ to 15 mM rapidly depolarized the membrane and no action potentials were elicited. The membrane potential reached a steady level of −40 mV. Addition of BAY K 8644 (1 μM) rapidly elicited several spikes (Fig. 1 *C*). The duration of the action potentials increased from 120 ms to >3 s. The amplitude also increased during exposure to the drug (from 12 to 26 mV). In the presence of 15 mM K^+, the undershoot following the larger spikes was less marked than in the presence of 5.6 mM glucose.

To determine the effects of BAY K 8644 at a membrane potential close to the potential at the peak of the spikes, which is around −15 mV (8), we increased K^+ from 5 to 50 mM (data not shown). Addition of BAY K 8644 (1 μM) after 5 min in 50 mM K^+ did not affect the membrane potential.

Effects of BAY K 8644 in the Presence of 11.1 mM Glucose

BAY K 8644 (1 μM) induced several modifications of the burst pattern of electrical activity recorded in the presence of 11.1 mM glucose (Fig. 2). After addition of the Ca^{2+}-channel agonist, the silent phase of the burst was transiently abolished and the membrane remained depolarized at the plateau potential. Spike activity was increased (Fig. 2 *A*). Then, the membrane spontaneously hyperpolarized and, after 3 min of exposure to BAY K 8644, the silent phase reached a new potential, ~10 mV more negative than in the absence of the drug. The duration of each burst was lengthened and the burst frequency was decreased from 4 to 3 bursts/min. Also, the duration of the silent phase was increased from an average of 4.59 ± 0.14 to 6.75 ± 0.41 s after addition of the drug.

Details of the electrical activity recorded before (Fig. 2 *B, a*), during (Fig. 2 *B, b*), and after (Fig. 2 *B, c*) exposure to BAY K 8644 (1 μM) are shown with an expanded time base in Fig. 2 *C*. In the presence of the drug, the number of spikes elicited during the active phase of the burst was dramatically reduced. However, the duration of the spikes was increased, lasting on occasion >1 s (compared with 0.08 s in the absence of the drug). Although the potential at the peak of the spikes was nearly constant (compare records in Fig. 2 *C*), the potential at the end of the repolarization phase of the spikes was 20–25 mV more negative than in control bursts, reaching a potential value similar to that of the silent phase of the burst. Also the shape of the spikes was affected (Fig. 2 *C*); this was even more obvious for the larger spikes. The spikes exhibited a biphasic, sawtooth-like ascending phase and a rapid descending phase.

After the removal of BAY K 8644, the potential of the silent phase slowly depolarized and, usually after ~10 min, reached a value similar to that recorded before the administration of the drug. A slight increase in the spike frequency was always recorded, but even 30 min after removal of BAY K 8644, the burst remained lengthened and the characteristics of the spikes altered (Fig. 2 *C*, compare *c* to *a*).

Finally, if the drug was added for a short period of time to an electrically silent cell, the subsequent addition of 11.1 mM glucose induced a bursting activity similar to that illustrated in Fig. 2.

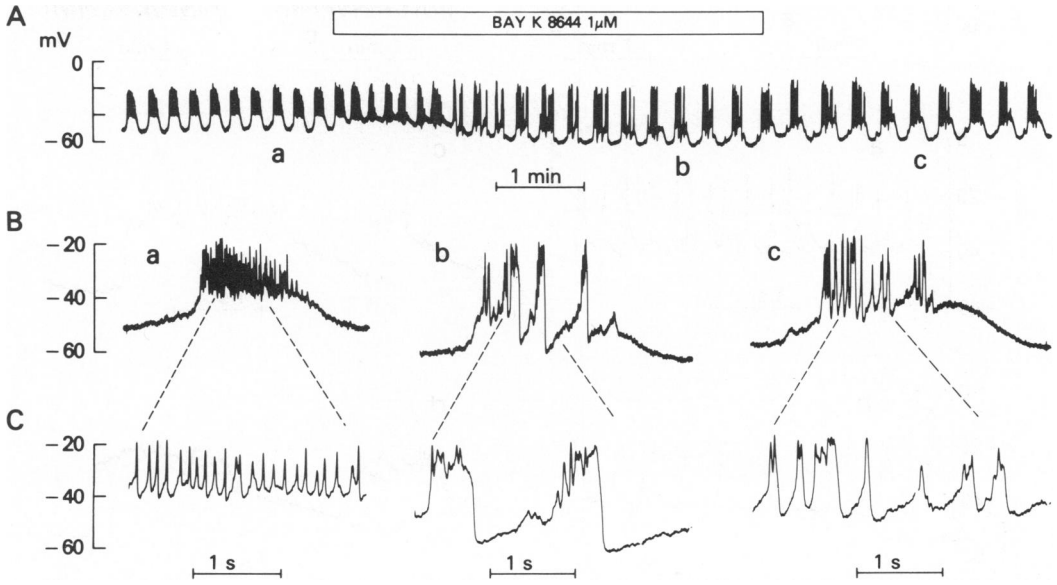

FIGURE 2 Effects of BAY K 8644 in the presence of 11.1 mM glucose. Effects of 1 μM BAY K 8644 on 11.1 mM glucose-induced electrical activity (*A*). Details of burst (*B*) and spikes from the same burst (*C*) recorded before (*a*), during (*b*), and after (*c*) addition of the drug.

Effects of Various Concentrations of BAY K 8644 on the Spike Activity Induced by High Glucose

At high glucose concentration, the membrane remained depolarized at the plateau potential and continuous spike activity was generated (Fig. 3 *A*), as previously described (20). The spike frequency, as well as the shape of the spikes elicited by 22.2 mM glucose, were fairly constant, although occasionally a spike showing a double peak was recorded (Fig. 3 *B, a*). Three concentrations of BAY K 8644 were tested. After addition of 100 nM BAY K 8644, the duration of the spikes was increased from 151.7 ± 3.3 to 314.5 ± 23.7 ms and the recording of a double or multiple peaked spike was a common occurrence (Fig. 3 *B, b*). The spike amplitude was not significantly affected, but the spike frequency was reduced from 3.92 to 2.43 spikes/s.

Raising the BAY K 8644 concentration to 500 nM or 1 μM, induced a further reduction in spike frequency and a further increase in duration. The ascending phase of the spike was biphasic (Fig. 3 *B, c* and *d*). The slow component arose from a hyperpolarized membrane potential value generated by the undershoot of the preceding action potential. It was generally observed that the larger the undershoot of the spike, the slower the initial part of the ascending phase of the following spike. As soon as this initial component reached a membrane potential value roughly equal to the threshold value from which the spike arose in the absence of the drug, a second steeper component appeared that rapidly brought the membrane potential to the peak value of the spike. The falling phase of the spikes was steeper. At the peak potential of the spikes, a succession of rapid fluctuations occurred, as also observed in the presence of 11.1 mM glucose (Fig. 2 *C, b*). These fluctuations were higher in the presence of 500 nM than in the presence of 1 μM BAY K 8644, but their amplitudes never exceeded 10 mV. The elongated spikes occurred in doublets, the second spike always lasting two to three times the duration of the first (Fig. 3 *B, c* and *d*). The effects of BAY K 8644 were irreversible, spike activity remaining altered 30 min after removal of the drug.

Effects of BAY K 8644 in the Presence of Quinine

To test the possibility that the effects of BAY K 8644 on the B-cell action potential could be mediated by modifications of the Ca_i^{2+}-activated K^+-channel, experiments were carried out in the presence of quinine, a drug known to block this channel in various cells, including the pancreatic B-cell (3). Addition of 100 μM quinine in the presence of 11.1 mM glucose abolished the burst pattern, inducing continuous spike activity (Fig. 4 *A, a* and *b*). Quinine also slightly increased spike duration (Fig. 4 *B, a* and *b*). Addition of BAY K 8644 (1 μM) in the presence of both 100 μM quinine and 11.1 mM glucose induced similar modifications as those described in the presence of 22.2 mM glucose. While the potential at the peak of the spikes

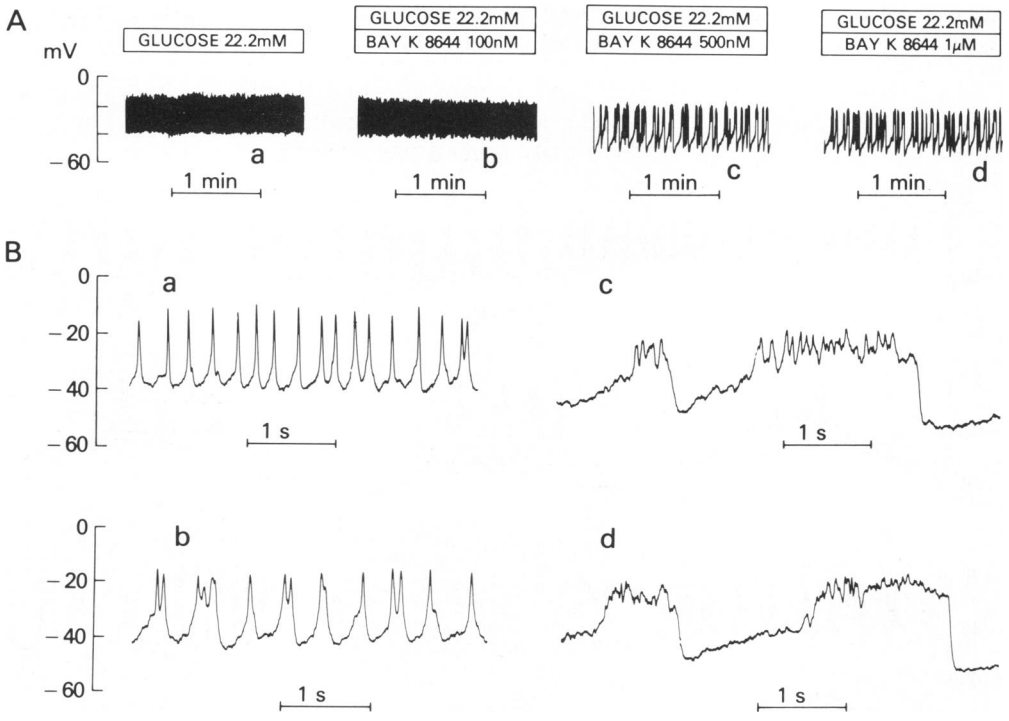

FIGURE 3 Effects of BAY K 8644 in the presence of 22.2 mM glucose. Effects of 100 nM, 500 nM, and 1 μM BAY K 8644 on 22.2 mM glucose-induced electrical activity (*A*). Details of spikes (*B*) recorded before (*a*) and during the presence of 100 nM (*b*), 500 nM (*c*), or 1 μM (*d*) BAY K 8644, as indicated under the traces in *A*. All records from the same experiment.

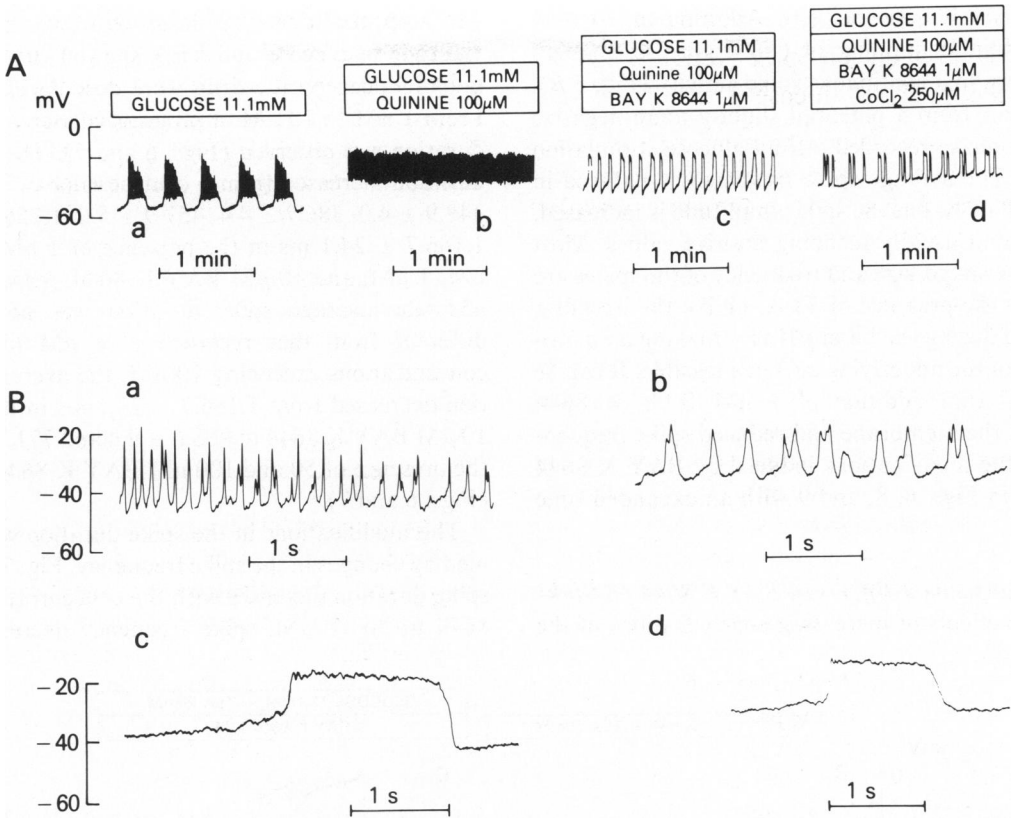

FIGURE 4 Effects of BAY K 8644 in the presence of glucose and quinine. B-Cell electrical activity recorded in the presence of 11.1 mM glucose (*A*, *a*) and after subsequent additions of 100 μM quinine (*A*, *b*), 1 μM BAY K 8644 (*A*, *c*), and 250 μM $CoCl_2$ (*A*, *d*). Details of spikes (*B*) recorded in *a*, *b*, *c*, and *d*. All records from the same experiment.

was unaffected, the spike frequency was reduced from 2.73 to 0.16 spikes/s. The duration of the spikes was 10 times longer than in the presence of both glucose and quinine and 30 times longer than in the presence of glucose alone (Fig. 4 *B*, *a–c*). The spikes were followed by an undershoot ~10 mV more negative than the potential at the foot of the spike.

Addition of Co^{2+} (250 μM $CoCl_2$), a Ca^{2+}-channel blocker, in the presence of BAY K 8644, partially reversed the modifications induced by the drug (Fig. 4 *B*, *d*). The spike duration was reduced from 2.09 ± 0.09 to 1.59 ± 0.13 s, while the spike frequency was slightly increased, from 0.16 to 0.18 spikes/s. Also, the spikes arose from a less negative membrane potential and their undershoots were almost completely abolished.

Effects of BAY K 8644 in the Presence of TEA

To better characterize the effects of BAY K 8644, experiments were carried out in the presence of TEA, a blocker of

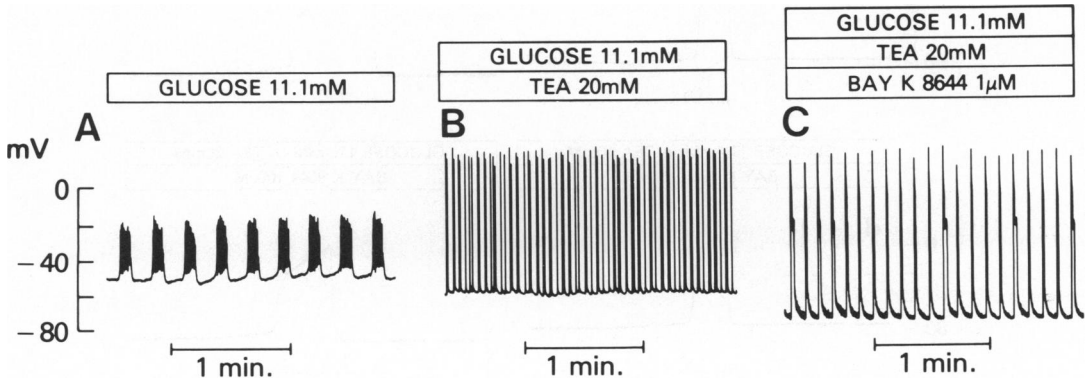

FIGURE 5 Effects of BAY K 8644 in the presence of glucose and TEA. B-cell electrical activity recorded in the presence of 11.1 mM glucose (*A*) and after subsequent additions of 20 mM TEA (*B*), and 1 μM BAY K 8644 (*C*). All records from the same experiment.

the voltage-gated K^+-channel (5). Addition of 20 mM TEA to a medium containing 11.1 mM glucose, changes the burst pattern to a continuous spike activity (Fig. 5 *B*). The spikes occur from a potential slightly more negative than the silent phase recorded during glucose stimulation without TEA. The average spike frequency is reduced in the presence of TEA, but the spike amplitude is increased, the peak potential usually reaching positive values. Most importantly, the shape, size and frequency of the spikes are very regular in the presence of TEA, unlike the irregular spikes recorded during the burst pattern, making a quantitative analysis of the underlying currents feasible. It can be seen in Fig. 5 that addition of 1 μM BAY K 8644 hyperpolarized the membrane and reduced spike frequency. Details of the modifications induced by BAY K 8644 are illustrated in Figs. 6, 8, and 9 with an expanded time base.

Dose-dependent Effects of BAY K 8644 on Spike Activity. The effects of increasing concentrations of the Ca^{2+}-channel agonist on the spike activity recorded in the presence of glucose and TEA showed that the modifications induced by the drug were dose-dependent (Fig. 6). From 1 nM to 10 μM, a progressive increase in the spike duration was observed (Figs. 6 and 7). The average spike duration increased from a control value of 322.5 ± 4.6 to 342.9 ± 4.0, 386.7 ± 4.4, 457.9 ± 5.0, 1,156.8 ± 77.9, and 1,186.7 ± 24.1 ms in the presence of 1 nM, 10 nM, 100 nM, 1 μM, and 10 μM BAY K 8644, respectively. At 10 μM, the average spike duration was not significantly different from that recorded at 1 μM ($P > 0.5$). At concentrations exceeding 10 μM, the average spike duration decreased from 1,186.7 ± 24.1 ms in the presence of 10 μM BAY K 8644 to 908.6 ± 9.4 and 771.7 ± 11.7 ms in the presence of 50 and 100 μM BAY K 8644, respectively (Figs. 6 and 7).

The modifications in the spike duration were accompanied by changes in the spike frequency. Fig. 7 shows that as spike duration increases with the concentration of BAY K 8644 up to 10 μM, spike frequency decreases. Further-

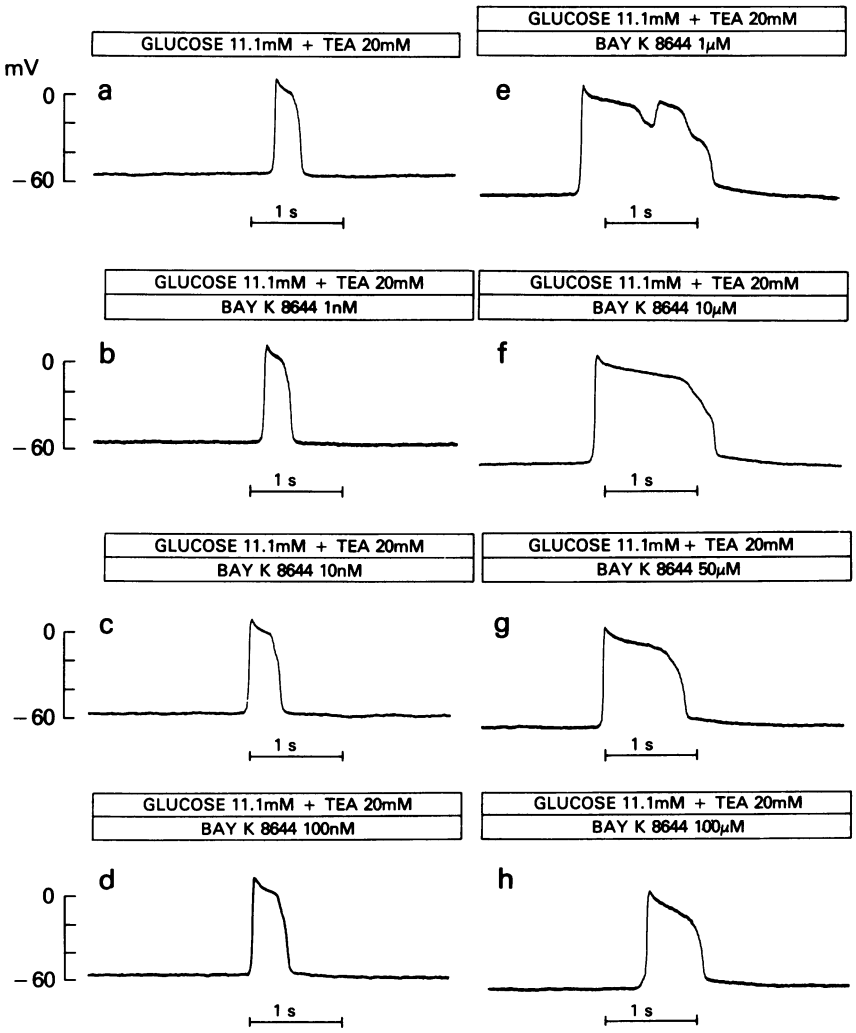

FIGURE 6 Spike duration at various BAY K 8644 concentrations. Effects of increasing concentrations of BAY K 8644 (as indicated above each record) on the spikes elicited by 11.1 mM glucose and 20 mM TEA. All records from the same experiment.

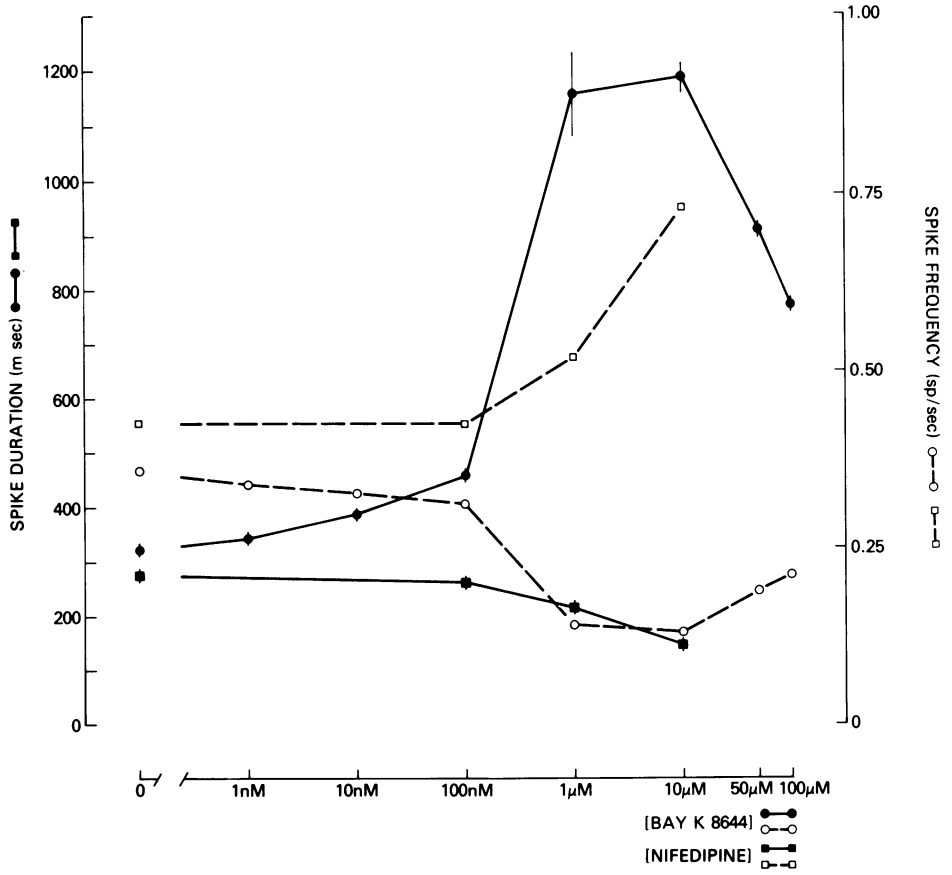

FIGURE 7 Dose-response curve for BAY K 8644 and Nifedipine in terms of spike duration and spike frequency. Spike duration (left ordinate, filled symbols) and spike frequency (right ordinate, open symbols) as a function of the concentration of BAY K 8644 (circles) and Nifedipine (squares). Glucose, 11.1 mM, and TEA, 20 mM, were present in all experiments.

more, the interval between the spikes was proportionate to the duration of the preceding spike. The ratio of the interval to the spike duration was constant at spike durations from 300 to 1,200 ms, at all concentrations of BAY K 8644 tested, and averaged 6.2 ± 0.4 (n = 364). Similar observations, but in the opposite direction, were made in the presence of the Ca-channel antagonist, Nifedipine, and are included for comparison in Fig. 7 (see Results, below, remarks on Fig. 10). It should be noted that the membrane potential recorded at the foot of the spikes was unchanged by BAY K 8644 concentrations from 1 nM to 100 nM but was 8–10 mV more negative at BAY K 8644 concentrations exceeding 100 nM. The spike amplitude was fairly constant at all concentrations tested.

Increasing BAY K 8644 concentrations from 1 nM to 1 μM induced a progressive increase in peak inward current. At concentrations exceeding 10 μM, the peak inward current was lower than the control value measured before the addition if BAY K 8644 Table I, A.

Effects of BAY K 8644 in the Presence of TTX and $CoCl_2$. To assess the specificity of BAY K 8644 in B-cells, we performed experiments in the presence of the Na^+-channel blocker, TTX, or the Ca^{2+}-channel blocker, Co^{2+}. The addition of TTX (1 μM) had no effect on the action potentials evoked by glucose and TEA (Fig. 8, *a* and *b*). Peak inward current averaged −17.42 ± 0.39 and −17.37 ± 0.55 pA before and after addition of TTX, respectively. In the presence of TTX, BAY K 8644 (1 μM) induced the same modifications of the action potential as in the absence of the Na^+-channel blocker; peak inward current (Table I, *B*), spike duration and membrane potential recorded at the foot of the spikes were increased (Fig. 8). Addition of TTX (1 μM) 5 min after the application of BAY K 8644 (1 μM) did not change the effects of the Ca^{2+}-agonist (data not shown).

On the other hand, the effects of BAY K 8644 were counteracted, in a dose-dependent manner, by the addition of Co^{2+}, a Ca^{2+}-channel blocker. Increasing the Co^{2+} concentration decreased the spike duration from 1,195.8 ± 51.5 ms (measured in the presence of 1 μM BAY K 8644 alone) to 773.3 ± 48.0, 429.1 ± 8.4, and 265.5 ± 6.6 ms in the presence of 100, 250, and 500 μM Co^{2+}, respectively. The increase in peak inward current elicited by BAY K 8644 was also antagonized by addition of Co^{2+} (Table I, *B*) In the presence of 1 mM Co^{2+} the spike activity was blocked and the membrane was depolarized by 11 mV (Fig. 8 *g*).

TABLE I
PEAK INWARD CURRENT (IN PICOAMPS)

A	TEA	TEA BAY 1 nM	TEA BAY 10 nM	TEA BAY 100 nM	TEA BAY 1 μM	TEA BAY 10 μM	TEA BAY 50 μM	TEA BAY 100 μM
	12.10 ± 0.26 $n = 22$	12.62 ± 0.33 $n = 23$	12.94 ± 0.32 $n = 21$	12.98 ± 0.31 $n = 22$	14.23 ± 0.53 $n = 14$	13.11 ± 0.21 $n = 18$	11.23 ± 0.24 $n = 18$	9.84 ± 0.09 $n = 14$
B	TEA	TEA TTX 1 μM	TEA TTX 1 μM BAY 1 μM	TEA TTX 1 μM BAY 1 μM Co^{2+} 100 μM	TEA TTX 1 μM BAY 1 μM Co^{2+} 250 μM	TEA TTX 1 μM BAY 1 μM Co^{2+} 500 μM		
	17.42 ± 0.39 $n = 13$	17.37 ± 0.55 $n = 9$	18.57 ± 0.11 $n = 15$	18.05 ± 0.07 $n = 15$	16.08 ± 0.59 $n = 7$	14.00 ± 0.31 $n = 7$		
C	TEA	TEA BAY 1 μM	TEA BAY 1 μM NIF 10 μM					
	15.78 ± 0.08 $n = 17$	16.89 ± 0.09 $n = 15$	8.77 ± 0.14 $n = 9$					
D	TEA	TEA NIF 100 nM	TEA NIF 1 μM	TEA NIF 10 μM				
	12.75 ± 0.10 $n = 13$	11.21 ± 0.23 $n = 10$	10.05 ± 0.11 $n = 11$	5.94 ± 0.07 $n = 15$				

To calculate the peak inward current, the B-cell membrane capacity was taken as 5 pF. Concentration of TEA was 20 mM throughout. Results are expressed as the mean (± SEM) with the number of individual spikes analyzed (*n*). *BAY*, BAY K 8644; *NIF*, Nifedipine.

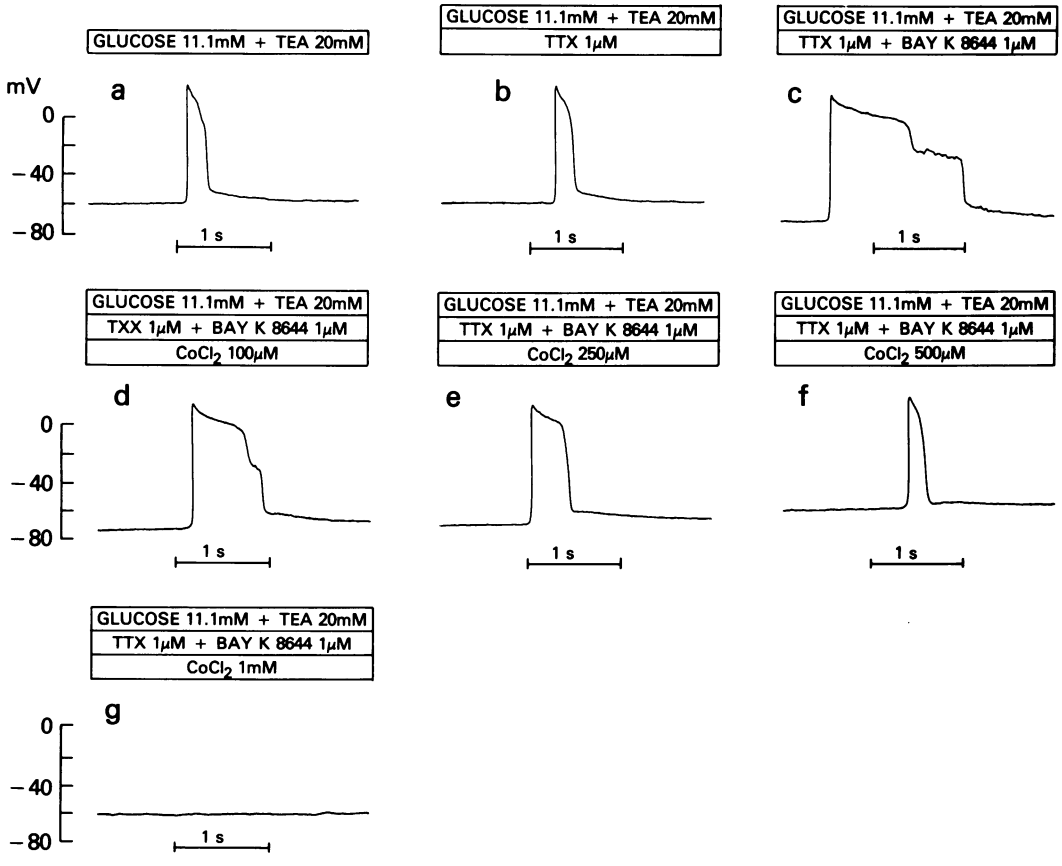

FIGURE 8 Antagonistic action of $CoCl_2$. Action potentials recorded in the presence of 11.1 mM glucose and 20 mM TEA (*a*) and after subsequent additions of TTX (*b*), BAY K 8644 (*c*), and $CoCl_2$ (*d*, *e*, *f*, and *g*) at the concentrations indicated above each record. All records from the same experiment.

Reversal of BAY K 8644 effects by Nifedipine. Nifedipine, an organic Ca^{2+}-channel antagonist (11, 12, 14), was tested in the presence of BAY K 8644 (1 μM). Addition of 10 μM Nifedipine rapidly suppressed the spike activity and progressively depolarized the B-cell membrane (Fig. 9 *A*). As the membrane depolarized (~15 mV), the spikes reappeared and the spike frequency increased progressively. The analysis of the spikes showed that the Ca^{2+}-antagonist reversed the modifications induced by BAY K 8644 (Fig. 9 *b*). In the presence of BAY K 8644, addition of Nifedipine reduced spike duration by 55%, spike amplitude by 38%, and peak inward current by 48% (Table I, *C*). Similar effects were recorded with 1 μM Nifedipine, except that the depolarization was only ~4 mV (data not shown).

After removal of Nifedipine, both the membrane potential at the foot of the spikes and the spike amplitude were increased, while the spike duration was lengthened. However, the effects of Nifedipine were never completely reversible (Fig. 9 *B*, *d*).

Dose-dependent Effects of Nifedipine on Spike Activity

For the purpose of comparison, Nifedipine was tested on the spike activity induced by glucose and TEA. Addition of Nifedipine (0.1–1 μM) did not affect the membrane potential at the foot and at the peak of the spike. However, addition of 10 μM Nifedipine transiently reduced spike frequency and the membrane potential at the foot of the spikes slowly decreased (Fig. 10 *A*, *d*). After the membrane depolarized ~16 mV, spike frequency again increased, but spike amplitude decreased. The duration of the spikes was decreased, from 274.5 ± 3.5 ms before addition of Nifedipine, to 260.4 ± 3.3, 214.0 ± 2.0, and 143.0 ± 1.8 ms in the presence of 100 nM, 1 μM, and 10 μM Nifedipine, respectively (see Figs. 7 and 10 *B*); peak inward current was reduced by 12%, 21%, and 53%, respectively (Table I, *D*). Spike frequency was not altered by 100 nM Nifedipine, but was increased, from 0.43 spikes/s before addition of Nifedipine, to 0.52 and 0.73 spikes/s, in the presence of 1 and 10 μM Nifedipine, respectively (Fig. 7). The interval between the spikes was proportionate to the duration of the spikes. The ratio of the interval to the spike duration was constant at spike durations from 140 to 275 ms, at all concentrations of Nifedipine tested, and averaged 8.1 ± 0.2 (n = 328).

The effects of Nifedipine were not reversed even 20 min after the removal of the Ca^{2+}-channel antagonist (Fig. 10 *A* and *B*, *e*).

DISCUSSION

BAY K 8644, a lipophylic dihydropyridine derivative, has been proposed to stimulate Ca^{2+} influx in different cell types (9, 10, 15, 19, 23, 27). Analysis of the drug effects at the level of single Ca^{2+}-channels revealed that the amplitude of the Ca^{2+}-channel unitary current was unaffected, but both mean open time and opening probability were increased (13, 16).

BAY K 8644 Is a B-Cell Ca^{2+}-Channel Agonist. In excitable membranes, the shape of the action potential is known to be determined primarily by the characteristics of the inward depolarizing and the outward repolarizing currents (14). Modifications of either of these two components will affect action potential shape.

In the present study we have shown that BAY K 8644 increased the maximum rate of depolarization and prolonged the spike duration, both parameters previously

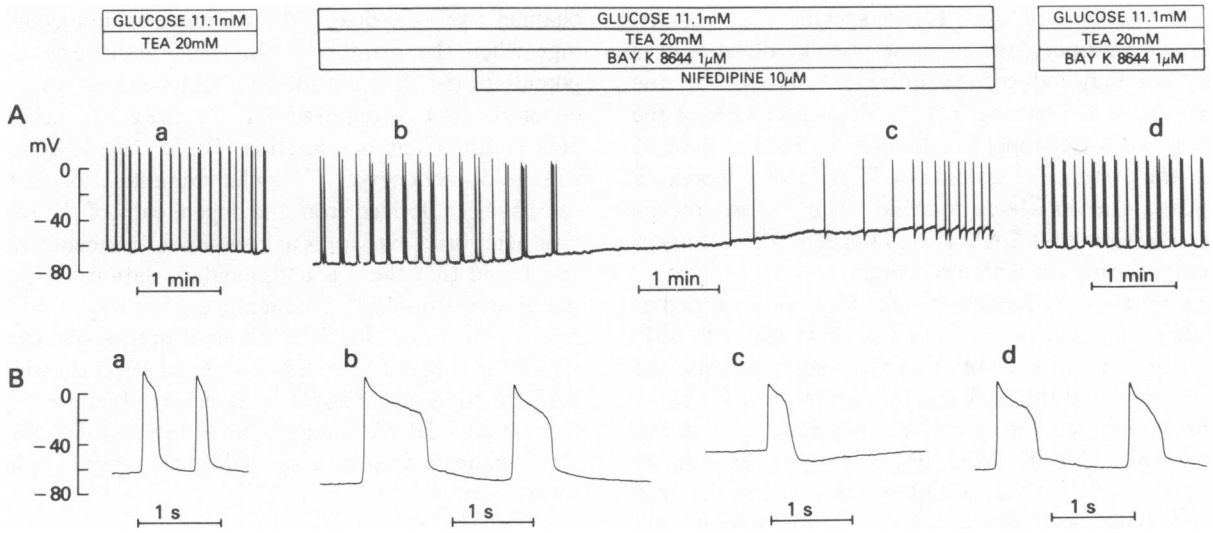

FIGURE 9 Antagonistic action of Nifedipine. Effects of addition of 1 μM BAY K 8644 and 10 μM Nifedipine on the electrical activity induced by 11.1 mM glucose and 20 mM TEA (*A*). Details of spikes (*B*) recorded in the presence of glucose and TEA (*a*), after addition of BAY K 8644 (*b*) or Nifedipine (*c*), and 10 min after removal of Nifedipine (*d*). All records from the same experiment.

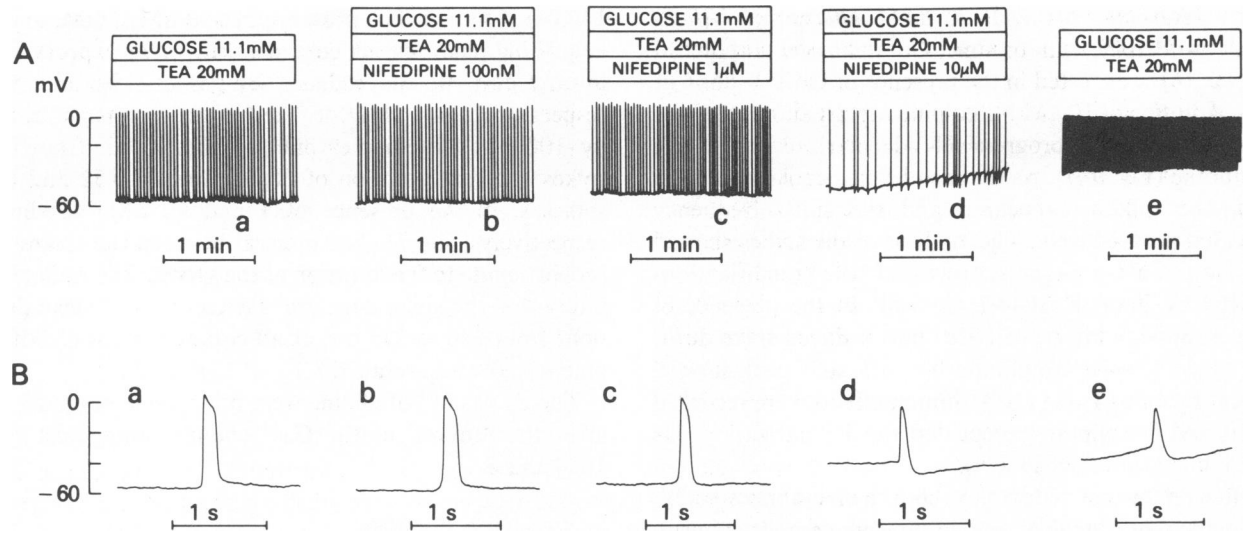

FIGURE 10 Effects of Nifedipine in the presence of glucose and TEA. Effects of increasing concentrations of Nifedipine (as indicated above the records) on the electrical activity induced by 11.1 mM glucose and 20 mM TEA (*A*). Details of spikes (*B*) recorded in the presence of glucose and TEA (*a*) or after the addition of Nifedipine (*b*, *c*, and *d*) and 5 min after removal of Nifedipine (*e*). All records from the same experiment.

shown to be Ca^{2+}-dependent (4, 24). Furthermore, cobalt, an inorganic Ca^{2+}-channel antagonist, and Nifedipine, an organic Ca^{2+}-channel antagonist, reversed the modifications of the B-cell action potential evoked by BAY K 8644, suggesting that the effects of BAY K 8644 are mediated by direct actions on B-cell Ca^{2+}-channels (17). Facilitation of Ca^{2+} influx by BAY K 8644 in pancreatic B-cells is further supported by recent observations showing that the drug enhanced the glucose-stimulated net uptake of ^{45}Ca; in the presence, but not in the absence, of extracellular calcium, BAY K 8644 stimulated ^{45}Ca outflow from rat pancreatic islets preloaded with ^{45}Ca (19).

The prolongation of the B-cell action potential and the increase in peak inward current induced by BAY K 8644 were not antagonized by TTX, a specific Na^+-channel blocker. Furthermore, the increase in spike duration was larger than that induced by quinine, a blocker of the Ca^{2+}-activated K^+-channel (3), or TEA, a blocker of the voltage-gated K^+-channel (5). Indeed, addition of BAY K 8644 in the presence of quinine or TEA further increased the spike duration about fourfold. Thus, these results support the view that BAY K 8644 specifically enhances Ca^{2+} entry during the action potential.

The progressive reduction of spike duration recorded at the higher concentrations of BAY K 8644 (50–100 μM) are probably due to a decrease in Ca^{2+} entry because the drug also inhibited the peak inward current over the same concentration range. Furthermore, it was previously shown that 87 μM BAY K 8644 decreased glucose-induced insulin release (19). Thus, at high concentrations, the drug may lose some specificity as a Ca^{2+}-channel agonist and could act as a dihydropyridine Ca^{2+}-channel antagonist (10).

Finally, the finding that in the presence of glucose and TEA the shape of the action potential and the derived inward current are not affected by TTX reinforces the view that Na^+-channel activation is not involved in the generation of action potentials in the normal B-cell.

BAY K 8644 Acts on the Open Ca^{2+}-channel in B-Cells. BAY K 8644 induced spike activity in the presence of either 5.6 mM glucose or 15 mM K^+, when the membrane potential was near −50 mV, but failed to induce electrical activity when the membrane potential of the cell was near −70 mV. These findings suggest that there is a threshold membrane potential for BAY K 8644 action in the B-cell. This is in good agreement with previous observations showing that, at the single Ca^{2+}-channel level, the drug did not induce spontaneous openings when the membrane was held at a very negative potential (16). It has also been observed that a moderate depolarization is required for the drug to manifest its potentiating effect on secretion (10, 15, 19). Recently, the voltage-dependency of Ca^{2+}-channel activation in the B-cell has been derived from the dependency of the recovery time after brief exposures to elevated K^+ concentrations. It was found that there is a sigmoidal relationship between the fraction of Ca^{2+}-conductance activation and membrane potential, with 50% channel activation occurring at −27 mV (8). The results presented here, showing that BAY K 8644 was ineffective at potentials more negative than about −50 mV, suggest that at least 10% of the B-cell Ca^{2+}-channels must be activated for the drug to induce an action potential.

Action Potential Duration Reflects Ca^{2+} Influx. BAY K 8644 has been reported to evoke a dose-dependent increase in the duration of the action potential

in new born rat heart cells that was paralleled by an increased rate of ^{45}Ca uptake and an increased contractility (23). Also, in adult guinea pig ventricular cells, the prolongation of the action potential evoked by various concentrations of BAY K 8644 was accompanied by a concomitant increase in maximum Ca current (6). The results presented here with BAY K 8644, Nifedipine, and cobalt also show a positive correlation between the spike duration and the peak inward current. Nifedipine, a selective Ca^{2+}-channel blocker, induced a dose-dependent decrease in peak inward current, which was accompanied by a concomitant shortening of the spikes. On the other hand, low concentrations of BAY K 8644 increased both peak inward current and spike duration.

In the presence of 11.1 or 22.2 mM of glucose, the elongated spikes elicited by BAY K 8644 were followed by an undershoot. This negative after-potential was reduced by quinine and, thus, appears to be due to stimulation of Ca_i^{2+}-activated K^+-channel. Furthermore, since the amplitude of the undershoot was also reduced by Co^{2+}, it can be considered as an indirect reflection of the entry of Ca^{2+} during the spikes. In all the experiments performed, the amplitude of the undershoot paralleled the spike duration: the longer the spike, the larger the undershoot. In the presence of TEA, the membrane potential recorded between the spikes also depends on the Ca^{2+}-activated K^+-channel. This potential was increased when the effects of BAY K 8644 on the spike duration were maximal and was decreased when the effects of Nifedipine on spike duration were maximal.

Thus, the present data suggest that under the present experimental conditions the modifications in spike duration parallel changes in Ca^{2+} influx. This is in agreement with previous work showing that BAY K 8644 stimulated the Nifedipine inhibited, both in a dose-dependent way, the glucose-stimulated insulin release (18, 19).

Intracellular Ca^{2+} May Inactivate Ca^{2+}-Channels in Pancreatic B-Cells. Among its numerous properties, the Ca^{2+}-channel has been proposed to exhibit some form of inactivation that is time- and, to some extent, voltage-dependent, but especially Ca_i^{2+}-dependent (12, 14, 22). According to this hypothesis, as Ca^{2+} ions flow during action potential, there is a local rise of intracellular free-calcium, which in turn causes inactivation of the Ca^{2+}-channel. The channel remains refractory until the internal free-calcium concentration is reduced.

The results reported here with either BAY K 8644 or Nifedipine could be explained by assuming the existence of such an inactivation process in the pancreatic B-cell. As discussed above, if the increase in spike duration reflects an increase in Ca^{2+} entry, then the increase in Ca_i^{2+} should inactivate the Ca^{2+}-channel and increase the refractory period. This should lengthen the interval between spikes and decrease spike frequency. Conversely, a decrease in Ca^{2+} entry during the spikes should shorten the duration of intervals between the spikes and, therefore, increase spike frequency. The present data confirm these expectations, namely, the dose-dependent changes in spike duration evoked by BAY K 8644 or Nifedipine were accompanied by parallel changes in the intervals between spikes (the ratio of the interspike interval to spike duration was constant) and by opposite modifications in the spike frequency (see Fig. 7). Since these modifications in spike duration and frequency were independent of changes in the membrane potential, they probably indicate the existence of a Ca_i^{2+}-dependent Ca^{2+}-channel inactivation process in the pancreatic B-cell.

Effects of BAY K 8644 on Glucose-induced Electrical Activity. Ca_i^{2+}-Activated K^+-channels have been proposed to be involved in the control of the pancreatic B-cell burst pattern of electrical activity. For example, increasing extracellular Ca^{2+} concentration increased the duration of the silent phase and decreased the frequency of the bursts; decreasing Ca^{2+} was shown to decrease both the maximum repolarization potential between the bursts and the duration of the intervals between them (4, 21, 25). Thus, the progressive hyperpolarization of the membrane potential at the silent phase of the burst as well as the increase in the relative duration of the silent phase and the decrease in burst periodicity induced by BAY K 8644 are consistent with the hypothesis that the burst activity is mainly governed by Ca_i^{2+}-activated K^+-channels.

Some details of the shape of the action potentials in the presence of BAY K 8644 deserve comment. The spikes exhibit a biphasic rising phase (see Fig. 2 *C*, *b* and Fig. 3 *B*, *c* and *d*). The initial slow component of the rising phase of the spike is probably caused by the Ca^{2+} load from the preceding spike. As Ca_i^{2+} is lowered, the membrane is depolarized and the threshold potential for the activation of Ca^{2+}-channels is reached, initiating the fast component of the rising phase. The membrane potential at which the fast component was initiated was not changed by BAY K 8644.

Another interesting feature of the spikes recorded in the presence of BAY K 8644 is the presence of small potential fluctuations (5–10 mV) along the plateau of the action potential. These potential fluctuations disappeared in the presence of quinine or TEA, when the potential at the plateau was more depolarized, and could reflect Ca^{2+}-channel bursting to open state. Ignoring changes in membrane resistance accompanying the recruitment of open Ca^{2+}-channels and taking the size of the elementary Ca^{2+}-channel voltage event in pancreatic B-cells as ~53 μV (2), these small potential fluctuations could be viewed as the synchronized opening of a few hundred Ca^{2+}-channels.

BAY K 8644 Affinity for the B-Cell Ca^{2+}-Channel. The existence of a dihydropyridine binding site or receptor closely associated with the calcium channel has

been reported in many tissues and its properties are consistent with its being a glycoprotein macromolecule (11). In our hands, the effects of BAY K 8644 were rapid in onset, but slowly reversible. This feature, and the finding that the drug exhibited an effect at very low concentrations, are in support of the existence of high affinity sites in the pancreatic B-cell.

On the other hand, although the onset of the effects of BAY K 8644 were voltage-dependent, its binding properties appeared to be voltage-independent. Indeed, the effects of BAY K 8644 could be evoked by depolarization after previous exposure of the hyperpolarized cells to the drug. This finding is consistent with the idea that the dihydropyridines, including BAY K 8644, which are highly lypophylic agents, may have access to their targets through the lipid phase of the membrane (6, 16).

In summary, electrophysiological evidence is provided for the first time in pancreatic B-cells that BAY K 8644, a new dihydropyridine drug shown to act as a Ca^{2+}-channel agonist in other systems, increases Ca^{2+} entry in response to depolarization of the B-cells. The data also provide the first indication for the existence of Ca_i^{2+}-induced Ca^{2+}-channel inactivation in the B-cell. The drug proves a useful tool in investigating the various repercussions on membrane potential that an increase of Ca^{2+} influx could have in B-cells within the intact islet.

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