

Activation of Ca Channels in Heart Muscle by a New Dihydropyridine Derivative

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1,4 dihydropyridines (e.g. nifedipine) undoubtedly are among the most powerful calcium antagonists. They act by blocking Ca channels when bound from the outside of the sarcolemma on a proteinic receptor (Glossmann et al. 1982). From inotropic measurements it had been supposed that a new dihydropyridine compound (BAY-K 8644, Methyl 1,4-Dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) binds to the same receptor as nifedipine but has diametrically opposite effects as compared to Ca antagonists, supposedly by stimulating Ca channels (Schramm et al. 1983a).

In the present work it was tried to check the hypothesis concerning the dihydropyridine-induced activation of Ca channels in the ventricular mammalian myocardium.

To estimate effects on the slow Ca inward current the TTX-insensitive Ca-mediated slow action potentials were measured after complete inactivation of Na channels by depolarization in elevated K^+ solutions (Carmeliet 1980). Slow action potentials (sAP) were elicited from right-ventricular papillary muscles of guinea pig in K^+ rich (18 to 40 mmol/l, isoosmolar reduction of NaCl) and 0.5 mmol/l $BaCl_2$ containing Tyrode solutions (in mmol/l: NaCl 150; $CaCl_2$ 2.5; $MgCl_2$ 0.5; tris-HCl 10; glucose 12; gassed with 100% O_2 ; $32 \pm 1^\circ C$, pH 7.32—7.37). sAP were measured using conventional 3 mol/l KCl-filled microelectrodes and differentiated by an active differentiator yielding the rate of rise (\dot{U}_{max}). The preparations were electrically driven (pacing interval between 1 and 10 s, voltage 1.2 times the threshold, duration 2—5 ms). BAY-K 8644 (BAY-K) was used in concentrations between 0.02 and 2.82 $\mu mol/l$. Only results from the same impalement were considered.

In solutions containing 18 mmol/l split \dot{U}_{max} can be recorded resulting from a mixed activation of residual fast channels and slow channels (Arita et al. 1983). BAY-K increased the second (slow channel) hump in partially depolarized papillary muscles. 5 minutes after switching of the BAY-K superfusion the effect on the second hump disappeared restoring split \dot{U}_{max} again (Fig. 1A). In 20 mmol/l KCl and 10^{-5} mol/l TTX, BAY-K reversibly increased both \dot{U}_{max} of sAP and the maximum overshoot potential \dot{U}_{max} of sAP and the maximum overshoot potential

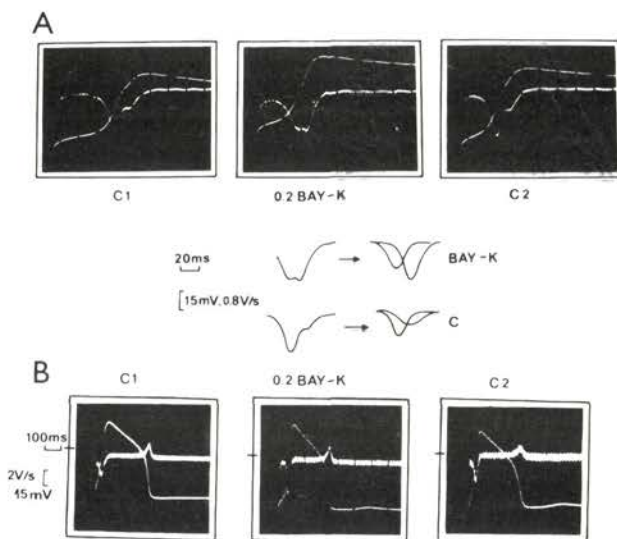


Fig. 1. A: Action of BAY-K on split \dot{U}_{max} in partially depolarized muscles (18 mmol/l K^+). The scheme indicates the possible composition of the split peak of \dot{U} from the residual fast (first) and slow channel response (see Arita et al. 1983). B: Effects of BAY-K on slow action potentials (20 mmol/l K^+ , 10^{-5} mol/l TTX, 0.2 μ mol/l BAY-K, pacing interval 2 s, C1: control solution before, C2: 8 min after switching off the BAY-K superfusion. Horizontal bars indicate zero potential in all figures.

(\dot{U}_{max} 3.5 ± 1.2 V/s (control), 7.7 ± 1.0 V/s (0.2 μ mol/l BAY-K), \dot{U}_{ov} 27 ± 3 mV (control), 33 ± 2 mV (BAY-K), $n = 4$) (Fig. 1B).

The effects of BAY-K on the duration of sAP were found to be crucially dependent on both the K^+ concentration and the pacing interval and they have not been considered in this context. The application of BAY-K was always accompanied by a small but significant hyperpolarization (-47 ± 3 mV (20 mmol/l KCl Tyrode solution), -52 ± 4 mV (20 mmol/l KCl Tyrode, 0.2 μ mol/l BAY-K)). In 20 mmol/l KCl maximum effects on \dot{U}_{max} were increased by $71 \pm 17\%$ ($n = 4$, effects were calculated by $100 \times (\dot{U}_T - U_C) / U_C$, where C means control conditions, T the application of 0.2 μ mol/l BAY-K, pacing interval 2 s).

In high K^+ media (40 mmol/l) only extremely small sAP could be evoked. Application of BAY-K dramatically restored the slow response activity. Over-shooting sAP could be recorded. \dot{U}_{max} was distinctly increased and sAP were prolonged at all pacing intervals (\dot{U}_{max} 0.9 ± 0 , V/s (control), 4.1 ± 0.9 V/s (0.4 μ mol/l BAY-K), U_{ov} 2 ± 5 mV (control), 27 ± 4 mV (0.42 μ mol/l BAY-K), maximum effects at pacing intervals of 10 s, $n = 3$). Only a small hyperpolarization could be elicited (U_r -34 ± 2 mV (control), -37 ± 2 mV (0.42 μ mol/l BAY-K,

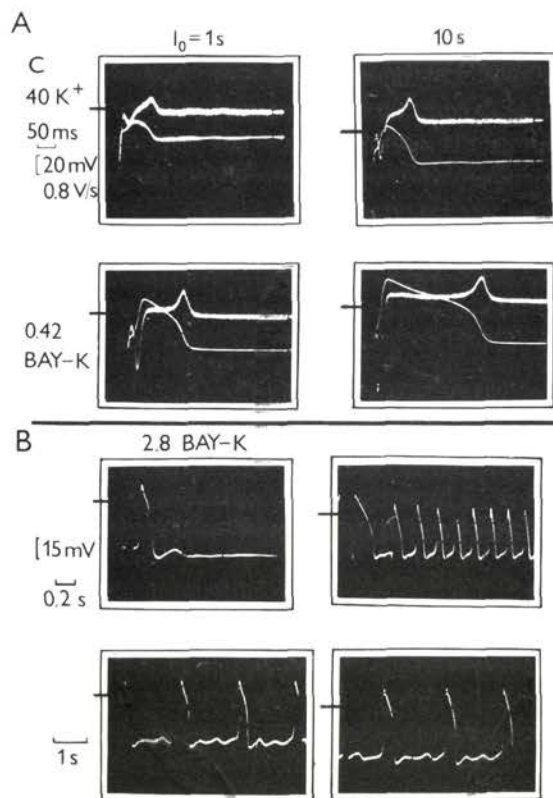


Fig. 2. A: Effects of BAY-K in extremely high K⁺ concentrations. Effects at two pacing intervals I_0 are demonstrated (obtained with the same cell). Effects were measured 15 minutes after the application of 0.42 $\mu\text{mol/l}$ BAY-K. B: BAY-K (2.8 $\mu\text{mol/l}$) evoked oscillatory afterdepolarizations resulting in severe dysrhythmia (30 mmol/l K⁺, initial pacing interval 5 s, effects measured 5; 8; 12; and 15 minutes after the application of the compound).

pacing interval 10 s, maximum effects, $n = 3$). All the above effects were found to be pacing dependent. The effectiveness of BAY-K increased in rapidly paced papillary muscles (maximum effects of 0.42 $\mu\text{mol/l}$ BAY-K on \dot{U}_{max} 442 \pm 37 % at 1 s, 177 \pm 23 % at 10 s pacing interval, Fig. 2A).

In concentrations of BAY-K exceeding approx. 1 $\mu\text{mol/l}$ all signs of an intracellular Ca overload could be observed: oscillatory after-depolarizations, triggered activity evoked by premature sAP, spontaneous activity evoked by a regular sAP as well as spontaneously active papillary muscles (Fig. 2B).

All the results obtained show that BAY-K acts in a stimulatory manner on the slow response activity regarded as an indicator of the slow channel activation in

cardiac muscle. The effects were not associated with any signs of reduction of K conductance which also could crucially increase \dot{U}_{\max} of sAP. Hyperpolarization almost unchanged maximum velocity of repolarization, increased effects in high K⁺ solutions and rapid pacing rates contradict a possible decrease in g_K . It is further known that BAY-K does not bind to other receptors such as muscarinic, α - and β -adrenergic, serotonin, GABA, histamine, diazepam and dopamine receptors (Schramm et al. 1983a, b). Thus, a direct stimulation of Ca channels (increase in the maximum conductance \bar{g}_{Ca} , a shift in the inactivation curve to the right, increase in the inactivation and activation time constants ratio) is the most convincing explanation of the increase in \dot{U}_{\max} of sAP. A further direct evidence of Ca channels being stimulated by BAY-K seems to be the ability of BAY-K to provoke an intracellular Ca overload as indicated by oscillatory afterdepolarizations (Kass et al. 1978; Matsuda et al. 1982), supposedly induced by Ca-induced activation of a large cation (transient inward) channel (Colquhoun et al. 1981).

The ability of the new dihydropyridine to modulate Ca channels, as proved by its effectiveness on sAP, suggests the existence of a certain class of molecules effectively interacting with the physiological Ca channel function.

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