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Impairment of Ca release from mammalian ventricular sarcoplasmic reticulum by the calcium channel agonist Bay K 8644

L.V. Hryshko & [1]D. Bose

Departments of Pharmacology & Therapeutics and Internal Medicine, University of Manitoba, Faculty of Medicine, Winnipeg, Manitoba, Canada, R3E 0W3

Positive inotropic concentration of the Ca-channel agonist, Bay K 8644, depressed contraction of canine right ventricular trabecula immediately after a rest period of 8 min, without decreasing action potential plateau amplitude. In contrast, high external Ca and ouabagenin caused only a slight decrease in post-rest contraction. Bay K 8644-induced post-rest depression was inversely related to the extracellular Ca concentration. Hence it could not be due to cellular Ca overload. Since post-rest potentiation is due to increased contribution of Ca from the sarcoplasmic reticulum, these results suggest that Bay K 8644 decreases the amount of releasable Ca from this structure during rest.

Introduction Increase in contractility of cardiac muscle after a brief period of rest (rest-potentiation) is due to an increase in the amount of Ca^{2+} released from the sarcoplasmic reticulum (Bers, 1985). The initial objective of this study was to examine the effect of the Ca-channel agonist, Bay K 8644 (Schramm *et al.*, 1983), on rest-potentiation in the canine ventricular myocardium. In the process we discovered an unexpected impairment of rest-potentiation, with this agent.

Methods Trabeculae (1–1.5 mm wide, 5 to 7 mm long) were obtained from the right ventricle of dogs (5–12 kg) of either sex, anaesthetized with Na pentobarbitone (30 mg kg^{-1}, i.v.). They were equilibrated in Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.4, Na_2HPO_4 1.7, $MgSO_4$ 1.2, $NaHCO_3$ 25 and dextrose 11) at 37°C and bubbled with 95% O_2 and 5% CO_2 to obtain a pH of 7.4. Isometric contractions at optimum resting length were obtained by electrical stimulation at a basic stimulus interval of 2 s. Transmembrane potential was measured with glass microelectrodes. The amplitude of the first beat following rest for 8 min was expressed as a percentage of the steady-state beat immediately preceding the rest period. The effects of the following agents on post-rest response were studied: racemic Bay K 8644 (Bayer), ouabagenin (Sigma) and Ca (Fisher). Paired *t* test was used to analyse statistical significance of difference in self controlled experiments with a single treatment. Duncan's multiple range test was used in experiments with multiple treatments. Results are expressed as means ± s.e. mean.

[1] Author for correspondence at: Department of Pharmacology and Therapeutics, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3.

Results Experimental preparations were obtained from 5 animals. Typical results are shown in Figure 1. Rest for 8 min caused a potentiation of the first post-rest contraction (123 ± 20% of control; $P < 0.01$).

Bay K 8644 (1 μM) increased the strength of the regular beats to 198 ± 19.4% of control ($P < 0.01$). However, the post-rest contraction was depressed to 36.1 ± 8.1% of the preceding regular contraction ($P < 0.01$). Tension recovered rapidly within 5–10 beats of restimulation. Transmembrane action

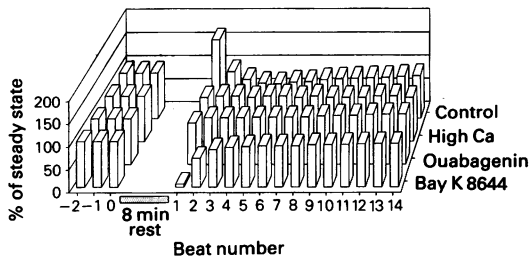

Figure 1 Typical post-rest contractions after 8 min rest and expressed as a percentage of the preceding regular contraction are shown. Separate muscle preparations were tested after no treatment (Control), or after treatment with increased extracellular Ca (5 mM), ouabagenin (1 μM) and Bay K 8644 (1 μM). The control data have been pooled from three tissues prior to treatment with the test agents. See text for explanation.

potential of the depressed contraction showed a prolonged duration and elevated plateau (not shown). Changing the extracellular Ca concentration altered the effect of Bay K 8644 on post-rest contractions. After rest for 8 min the contraction was $18 \pm 8.1\%$ of control in the presence of 1 mM Ca and Bay K 8644 and $63.8 \pm 7.6\%$ in the presence of 5 mM Ca and Bay K 8644 as compared to $36.1 \pm 8.1\%$ in the presence of the normal 2.5 mM extracellular Ca and Bay K 8644. These changes were significantly different from one another ($P < 0.05$).

Other inotropes were tested in concentrations which produced inotropic responses comparable to Bay K 8644. High extracellular Ca (5 mM) or ouabagenin (1 μM), caused only a slight reduction in the size of the post-rest beat.

Discussion Marked depression of post-rest contraction was an unexpected finding with Bay K 8644. This agent causes an increase in Ca current (Thomas *et al.*, 1985a). No effect has been observed on the sarcoplasmic reticulum (SR) in skinned muscle preparations (Thomas *et al.*, 1985b). Post-rest potentiation has been attributed to increased release of Ca from the SR (Bose *et al.*, 1984). This is because the amplitude of the action potential plateau of the potentiated post-rest beat is smaller than normal. Furthermore, post-rest potentiation is affected less by Ca channel blockers than regularly paced contractions (Bers, 1985). The effect of Bay K 8644 on post-rest contractions therefore seems to be due to a decrease in the size of the Ca pool in the SR or its release and not due to a decrease in inward Ca current because the action potential plateau amplitude is increased. It is also not due to the presence of the Ca channel blocking enantiomer in the racemic mixture because the pure Ca channel agonist enantiomer of Bay K 8644 also causes post-rest depression (Bose, unpublished observation). Excessive intracellular Ca overloading is believed to impair contractility by either interfering with cellular energy production (Lin & Vassalle, 1983) or by increasing spontaneous Ca release from the SR during diastole (Allen *et al.*, 1985). These are unlikely factors in the case of Bay K 8644-induced rest-depression because elevation of extracellular Ca decreased rest depression. The apparent decrease in post-rest potentiation by high extracellular Ca and ouabagenin may be due to the preparation approaching an inotropic ceiling and not due to any substantial effect on the SR. Decrease in rest-potentiation with long rest-intervals has been suggested to be due to an actual loss of Ca from the SR and eventually out of the cell (Bridge, 1986). This may also apply to Bay K 8644. In a previous study Thomas *et al.* (1985a) found no effect of Bay K 8644 on the SR-dependent rested state contraction of the guinea-pig atrium but this may be due to ultrastructural differences (e.g. absence of t-tubule in the atrium; Sommer & Johnson, 1979) which may minimize Ca loss from the SR during diastole.

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