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The positive inotropic action of the nifedipine analogue, Bay K 8644, in guinea-pig and rat isolated cardiac preparations

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1 The inotropic effect of Bay K 8644 has been studied in rat and guinea-pig atria and ventricular strips stimulated at 1 Hz, in a medium containing $CaCl_2$ 1.8 mM. The positive inotropic effect at maximal effective concentrations of Bay K 8644 was in the following order: guinea-pig ventricle > rat ventricle > guinea-pig atria >> rat atria.

2 In rat preparations, the tension recorded at maximum effective concentrations of Bay K 8644 was similar at three different calcium concentrations (0.7, 1.8, 3.0 mM). The amplitude of the positive inotropic effect evoked by Bay K 8644 increased when atrial and ventricular contractions were reduced by lowering the external calcium concentration.

3 The contractile tension reached in the presence of maximum effective concentrations of Bay K 8644 ($3 \times 10^{-7} - 1 \times 10^{-6}$ M) was greater than that produced by the maximum effective concentration of external calcium (3 mM) in rat ventricles but not in rat atria.

4 High doses of nifedipine ($3 \times 10^{-7} - 1 \times 10^{-6}$ M) depressed the contraction of rat atria more than the contraction of rat ventricles.

5 In rat ventricles, nifedipine shifted to the right the inotropic dose-effect curve of Bay K 8644.

6 It is concluded that the interaction between nifedipine and Bay K 8644 occurred at the same binding sites. These sites have some characteristics of the low affinity binding sites of nifedipine and other related dihydropyridines.

Introduction

Bay K 8644 is a nifedipine derivative which presents many actions opposite to those of the parent compound. It increases the contractile activity of guinea-pig cardiac preparations and evokes the constriction of rabbit isolated aorta by a direct effect (Schramm *et al.*, 1983). Bellemann (1984) has shown that Bay K 8644 interacts with other dihydropyridines for the same binding sites in beating myocytes. High affinity dihydropyridine binding sites have been identified in cardiac microsomal membranes (Bellemann *et al.*, 1981) and in brain, lung and kidney microsomal membranes (Bellemann *et al.*, 1983). Binding to these sites occurs at concentrations much lower than those required to observe a pharmacological effect in cardiac muscle (Triggle, 1982; Janis & Scriabine, 1983; Lee & Tsien, 1983), but not in the coronary artery (Godfraind *et al.*, 1984a,b). It has been shown that Bay K 8644 interacts functionally with calcium channels in cardiac (Bellemann, 1984) and smooth muscle (Godfraind *et al.*, 1984a), allowing the opening of the channels. It is considered as a calcium channel agonist (Towart & Schramm, 1984): in cardiac cells it appears to enhance calcium influx (Wahler & Sperelakis, 1984; Hess *et al.*, 1984) by prolongation of calcium channel open state (Kokubun & Reuter, 1984; Ochi *et al.*, 1984).

We have studied the inotropic effect of Bay K 8644 in various isolated myocardial preparations. Preliminary results have been presented previously (Finet *et al.*, 1984).

Methods

Wistar rats weighing 250–300 g and albino guinea-pigs (Dunkin Hartley) weighing 300–400 g were killed by decapitation, their chests opened and their hearts rapidly removed. Whole left atria and right ventricular

strips (approximate length 10 mm, width 1.5 mm) cut perpendicularly to the axis of the heart were dissected and suspended in organ baths under an initial resting tension (diastolic) of 500 mg in Tyrode solution containing (mM): NaCl 137, KCl 6, $CaCl_2$ 1.82, $MgCl_2$ 1.0, NaH_2PO_4 0.417, $NaHCO_3$ 11.5 and glucose 5.5. It was equilibrated at 30°C with a mixture of 95% O_2 and 5% CO_2. Before drug addition the Tyrode solution was changed every 15 min. The tissues were stimulated by field electrodes with rectangular 10 ms pulses (strength at least twice threshold) at different frequencies. Recordings of the contractile activity were made isometrically. After an initial equilibration period of either 45 min (atria) or 90 min (ventricles), Bay K 8644 was added cumulatively to the bath in order to obtain concentration-effect curves.

In some experiments, nifedipine was added before Bay K 8644. In experiments performed with varied calcium concentrations, the preparations were equilibrated in Tyrode containing calcium 1.8 mM after which the solution was changed for a low calcium (0.5 mM) Tyrode solution; then, the calcium was added cumulatively.

For each concentration of the drug, results have been calculated at the time of maximum effect, expressed either as a percentage of the initial systolic tension or as a force (in g). Drug effects were compared with the level of contraction maintained in control tissues incubated for the same time with appropriate solvent dilutions (acetone in Tyrode for dihydropyridine experiments). Experiments with nifedipine were conducted in a darkened room, using a Na lamp, in order to avoid drug degradation induced by daylight. Data are expressed as means ± s.e.mean.

Drugs

Bay K 8644 and nifedipine (Bayer, Leverkusen, FRG) were dissolved in acetone as stock solutions of 1×10^{-2} M and diluted before use in warm physiological solution.

Results

Calcium 1.8 mM

The control systolic contraction of rat right ventricular strips diminished when the rate of stimulation frequency was increased from 1 to 3 Hz. At 1 Hz, Bay K 8644 increased the ventricular systolic contraction in a concentration-dependent manner. At each concentration, the onset of the inotropic effect was observed for a few seconds after addition of the drug to the bath. The augmentation of the contraction was progressive with time and was sustained once the peak effect had been reached (after 10 to 20 min respectively for high and low doses).

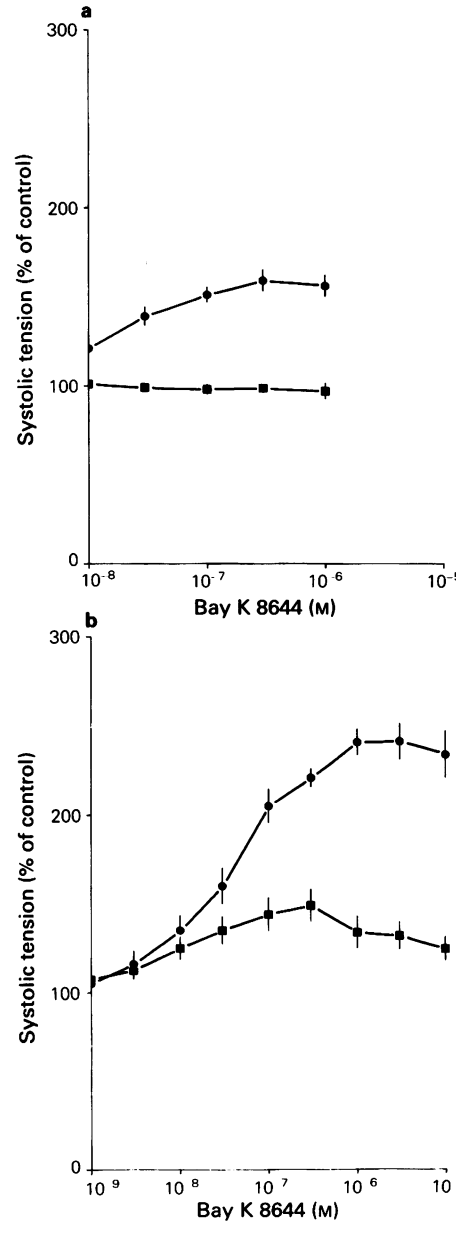

Figure 1 Systolic tension of rat (a) and guinea-pig (b) isolated cardiac preparations in presence of various doses of Bay K 8644. Left atria and right ventricular strips were incubated at 30°C in a physiological solution containing 1.8 mM calcium and electrically driven at a rate of 1 Hz. The addition of Bay K 8644 to rat (a) and guinea-pig (b) ventricular (●) and atrial (■) preparations was cumulative. Each curve is the mean of 15 experiments for rat ventricles, of 11 for rat atria and of 4 for guinea-pig preparations. Vertical lines indicate standard errors where they exceed the size of the symbol.

The maximum inotropic effect was obtained with Bay K 8644 3×10^{-7} M and corresponded to a $65 \pm 5\%$ ($n = 15$) increase of contraction (Figure 1a). Within the range of Bay K 8644 concentrations from 10^{-8} to 10^{-6} M, no change in ventricular diastolic tension was observed.

At 1 Hz, rat atria showed small changes in contractility after the addition of Bay K 8644 3×10^{-7} M.

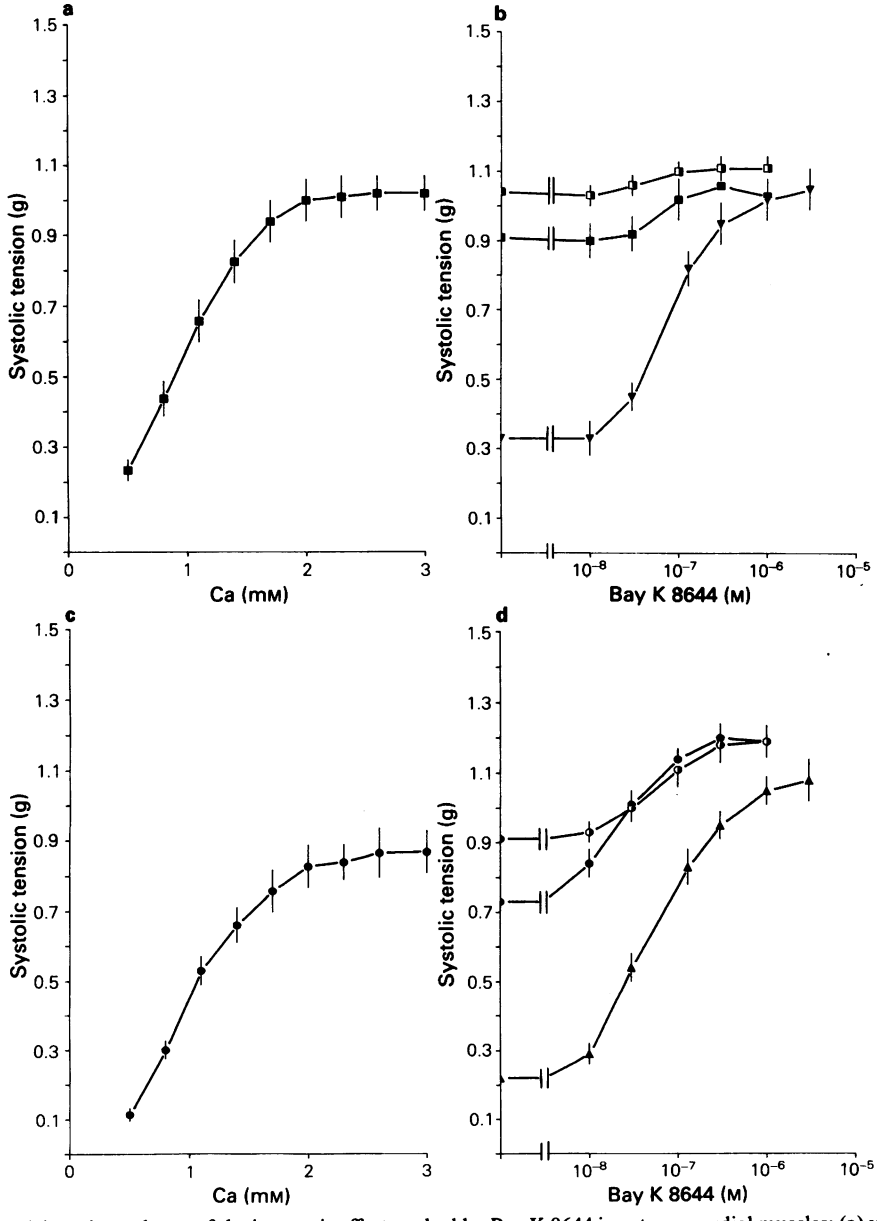

Figure 2 Calcium dependency of the inotropic effect evoked by Bay K 8644 in rat myocardial muscles: (a) variations of atrial systolic tension as a function of the external calcium concentration ($n = 8$). (b) Variations of atrial systolic tension with Bay K 8644 in a medium containing 0.7 (▼; $n = 7$), 1.8 (■; $n = 11$) and 3.0 mM calcium (◨; $n = 7$). (c) Variations of ventricular systolic tension in function of the external calcium concentration ($n = 8$). (d) Variations of ventricular systolic tension with Bay K 8644 in a medium containing 0.7 (▲; $n = 7$), 1.8 (●; $n = 11$) and 3.0 mM calcium (◑; $n = 7$).

Some preparations did not respond, others showed a weak increase in systolic tension; the maximum effect corresponded to an increase of $16 \pm 5{,}7\%$ ($n = 11$) with Bay K 8644, 3×10^{-7} M (Figure 1a). No variation in the atrial diastolic tension was seen after the addition of increasing doses of Bay K 8644.

At 1 Hz, the control systolic contractions of guinea-pig ventricular strips and atria were respectively equal to 0.41 ± 0.069 g and 0.89 ± 0.15 g and were not modified when the rate of stimulation was increased up to 3.3 Hz. We have therefore stimulated the guinea-pig myocardial tissues at the frequency used for the rat. In guinea-pig atria, the positive inotropic action of Bay K 8644 was apparent down to 1×10^{-9} M, the maximum inotropic effect was reached at 3×10^{-7} M and corresponded to $49 \pm 9\%$ increase in systolic contraction. The ED_{50} values was equal to $1 \pm 0.4 \times 10^{-8}$ M. When Bay K 8644 concentrations were increased above 3×10^{-7} M, the inotropic effect declined as a function of the concentration (Figure 1b).

In ventricular strips, the maximum positive inotropic effect of Bay K 8644 was observed for 1×10^{-6} M and corresponded to $141.46 \pm 10\%$ increase in systolic contraction. The threshold concentration was down to 1×10^{-9} M and the ED_{50} value was $3.5 \pm 0.7 \times 10^{-8}$ M. (Figure 1b). Within the range of Bay K 8644 concentrations studied, no change in ventricular and atrial diastolic tension was observed.

Calcium deprivation and excess

We have examined the inotropic effect of Bay K 8644 in preparations bathed in different calcium concentrations. In rat preparations, the contractile force was dependent upon $CaCl_2$ concentration and the maximum contraction was recorded when the concentration was close to 2.5 mM (Figure 2a). In guinea-pig preparations, arrhythmias were observed in most preparations when the $CaCl_2$ concentration was below 0.8 and higher than 11 mM concentration, for which the maximum contraction was not reached in this species. Therefore, we have not studied the effect of various calcium concentrations in guinea-pig preparations.

Figure 2 shows that an inotropic effect of Bay K 8644 was obvious in ventricles for each concentration of calcium so far examined; in the presence of the dihydropyridine, the maximum contractile tension recorded was higher than the maximum contraction evoked by $CaCl_2$ ($P < 0.01$). On the other hand, an inotropic effect was substantial in atria only when the medium contained $CaCl_2$ 0.7 mM and the maximum effect observed just reached the level of the contractility recorded at the maximum effective concentration of calcium.

From dose-effect curves obtained at 0.7 mM $CaCl_2$,

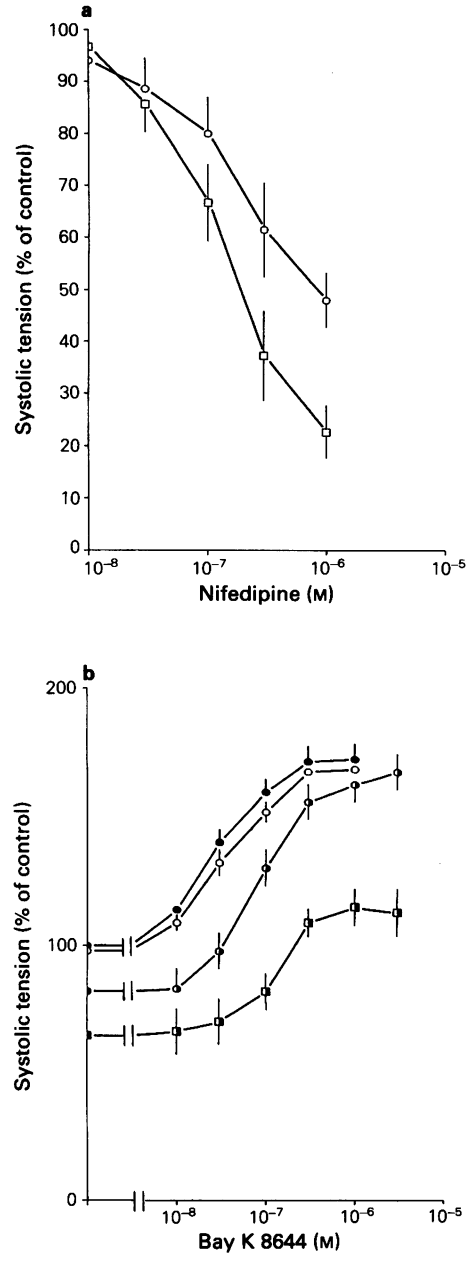

Figure 3 Variations of systolic tension with nifedipine (a) in rat isolated cardiac preparations and with Bay K 8644 in presence of nifedipine (b). (a) The addition of nifedipine to ventricular (O) and atrial (□) preparations was cumulative. Each curve is the mean of 4 experiments, vertical lines show s.e.mean. (b) Bay K 8644 cumulative doses in rat ventricles (control ●; $n = 4$), in presence of nifedipine 10^{-8} M (O; $n = 4$), 1×10^{-7} M (◑; $n = 4$) and 1×10^{-6} M. (■; $n = 4$).

Bay K 8644 ED_{50} values were calculated, they were respectively $6.3 \pm 1.2 \times 10^{-8}$ M and to $4.3 \pm 1.4 \times 10^{-8}$ M for atria and ventricles; this difference was not significant.

Nifedipine

These experiments were performed on rat atria and ventricles bathed in physiological solution containing $CaCl_2$ 1.8 mM. As Figure 3a illustrates, nifedipine depressed the contraction of the preparations in a dose-dependent manner. Atria were more depressed than ventricles; for nifedipine 1×10^{-6} M this difference was significant ($0.025 < P < 0.001$).

As shown in Figure 3b, dose-effect curves of Bay K 8644 in rat ventricles were displaced to the right in the presence of nifedipine 1×10^{-8} and 1×10^{-7} M; with nifedipine 1×10^{-6} M, there was also a depression of the maximum. In order to obtain an estimate of nifedipine affinity in this tissue, pA_2 was calculated according to Arunlakshana & Schild (1959). pA_2 was equal to 7.3 with nifedipine 1×10^{-7} M and to 7.5 with nifedipine 1×10^{-6} M.

Discussion

The experimental results described here confirm that Bay K 8644 has a positive inotropic effect in isolated cardiac preparations. In addition, they show that the efficacy of this drug is dependent on the nature of the preparation, since for $CaCl_2$ 1.8 mM, the order of the maximum response is as follows: guinea-pig ventricle > rat ventricle > guinea-pig atria >> rat atria. The inotropic effect of this drug also depends upon the concentration of calcium in the perfusion fluid.

When the extracellular calcium concentration was low enough to observe a significant inotropic effect, the ED_{50} values were similar in rat ventricles and atria (close to 5×10^{-8} M). From studies of the binding of Bay K 8644 to intact cardiac cells (Belleman, 1984) or to microsomal preparations of cardiac tissue (Vaghy *et al.*, 1984), a K_D value close to 4×10^{-8} M has been obtained. It appears that there is a good agreement between binding and positive inotropic effect of Bay K 8644. As quoted in the introduction, this is not apparently the case for the binding and negative inotropic effect of nifedipine and related dihydropyridines since various authors have found a high affinity binding in the 0.1 nanomolar range at which there is no reported effect of dihydropyridines on cardiac contractility (Triggle, 1982; Janis & Scriabine, 1983; Lee & Tsien, 1983; Godfraind *et al.*, 1984b; Vaghy *et al.*, 1984). Nevertheless some authors have reported the existence of a low affinity binding site with K_D values ranging around 5×10^{-8} M (Belleman, 1981; Marsh *et al.*, 1984).

The present observations with nifedipine indicate the existence of interactions between Bay K 8644 and nifedipine. We have observed that Bay K 8644 reversed the negative inotropic effect of nifedipine 1×10^{-8} and 1×10^{-7} M but not 1×10^{-6} M. A calcium antagonistic effect has been reported with high doses of Bay K 8644 (Hess *et al.*, 1984; Sanguinetti *et al.*, 1984; Vaghy *et al.*, 1984). The depression of the maximum inotropic effect of Bay K 8644 observed with nifedipine 1×10^{-6} M could tentatively be accounted for, by assuming a possible summation of the negative inotropic actions of the two compounds when high concentrations of both are used. The dose-effect curves of Bay K 8644 were shifted to the right by nifedipine and this shift was dose-dependent at the three doses studied. If we assume that this displacement observed by measuring contraction is due to the competition reported to exist in binding studies, then the value of pA_2 calculated here could be taken as an estimate of the affinity of nifedipine for its binding sites. Biological and radiochemical estimates of K_D value of nifedipine are of the same magnitude, close to 5×10^{-8} M. This indicates that competition between Bay K 8644 and nifedipine could occur at low affinity binding sites. It therefore appears that I_{50} (the concentration producing a 50% reduction in contractility) is not an estimate of the apparent dissociation constant of nifedipine from its binding sites. The magnitude of I_{50} values depends upon the affinity of nifedipine for its receptors and upon another factor. Our data show that the rat atria were more sensitive to the negative inotropic effect of nifedipine than were rat ventricles. It was also found that the positive inotropic efficacy of Bay K 8644 was higher in ventricles than in atria. It has been shown by Hess *et al.* (1984) that Bay K 8644 enhances Ca channel current by promoting a mode of gating behaviour characterized by a high probability of channel openness and a relatively long-lasting channel opening. This is in agreement with the observation that even when ventricles were maximally activated by calcium, Bay K 8644 caused a further positive inotropic effect. This model implies that calcium influx depends upon the number of channels available at each time. In view of the differences reported above between atria and ventricles, it may be assumed that in the presence of a given concentration of dihydropyridine, the number of open channels per unit of time could be different between these tissues. The actual experimental observations do not provide information either on a possible difference in calcium channel density or on a difference in dihydropyridine affinity between these tissues. There are, indeed, unexplained differences in B_{max} values reported for the binding of dihydropyridines to different tissues (Vaghy *et al.*, 1984). Such differences could depend upon the density of calcium channels but they need to be examined in atria and ventricles.

In conclusion the experiments described here indicate that the positive inotropic dihydropyridine Bay K 8644 has pharmacological actions related to its interaction with binding sites on calcium channels. These sites have some characteristics of the low affinity binding sites of nifedipine and other related dihydropyridines.

M.F. is Aspirant F.N.R.S.

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