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The effects of Bay K 8644 and nifedipine on the neural responses of the rabbit ear artery

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1 The effects of Bay K 8644 and nifedipine on the electrical and mechanical responses of the distal ear artery of the rabbit to stimulation of the perivascular nerves were studied.

2 Neural stimulation elicited excitatory junction potentials (e.j.ps) in the smooth muscle cells of the artery. An action potential was generated when the e.j.p. reached a threshold of about -53 mV. Contraction was always triggered by the action potential.

3 Bay K 8644 at low concentrations (7×10^{-8} M) had no significant effect on the resting membrane potential and the e.j.p. However the amplitudes of the action potential, and the contraction were potentiated by Bay K 8644. Multiple action potentials could also be generated from a single e.j.p.

4 At higher concentrations, Bay K 8644 (3×10^{-6} M) caused membrane depolarization, development of vascular tone and spontaneous action potentials. All the effects of Bay K 8644 could be reversed by exposure to light.

5 Nifedipine (3×10^{-7} M) inhibited the action potential and the resulting contraction. The effect of nifedipine could be reversed by Bay K 8644 but not by light.

6 These results demonstrate that the excitatory and inhibitory effects of Bay K 8644 and nifedipine on vascular smooth muscle are associated with changes in the electrical responses.

Introduction

Two types of electrical response to stimulation of the perivascular nerves have been identified in vascular smooth muscle cells – a fast depolarization known as the excitatory junction potential (e.j.p.) and a slow depolarization (Cheung, 1982; 1984). When the excitatory junction potential is large enough to reach a certain threshold, an action potential is triggered, resulting in a fast twitch-like contraction (Cheung, 1984). Since it is known that calcium is the ion that carries the inward current of the action potential in most smooth muscle cells, it would be expected that agents that interact with the calcium channels such as the calcium agonist Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) and the calcium antagonist nifedipine would have profound effects on the action potential and the resulting contraction in vascular smooth muscle. In cardiac muscle cells, Bay K 8644 prolongs the duration of the action potential (Brown *et al.*, 1984; Thomas *et al.*, 1985), increases the probability of opening of single calcium channels (Brown *et al.*, 1984) and prolongs the mean opening time of calcium channels (Kokubun & Reuter, 1984; Hess *et al.*, 1984). The electrophysiological effects of Bay K 8644 on vascular smooth muscle have not been studied. Hence, in the present study, we investigated the effects of Bay K 8644 and nifedipine on nerve-evoked electrical and mechanical activities of the rabbit ear artery.

Methods

Young New Zealand rabbits 1–1.5 kg were used. Segments about 3 mm in length were taken from the distal end of the central ear artery. The experimental set-up and procedures were similar to those previously described (Cheung, 1984, 1985a). Tension development in ring segments of artery was measured by two fine tungsten wires inserted through the lumen. One wire was connected to a Narco F60 force transducer while the other served as an anchor. A resting tension of about 250 mg was applied in all preparations. For stimulation of perivascular nerves, single pulses of 0.1 ms duration from a Grass S48 stimulator were used. The temperature of the preparation was main-

tained at about 36°C by continuous superfusion with a physiological solution (composition, mM: NaCl 120, $NaHCO_3$ 25, glucose 11, KCl 5, $CaCl_2 2H_2O$ 2.5, $NaH_2PO_4 H_2O$ 1 and $MgSO_4 7H_2O$ 1, bubbled with 95% O_2 and 5% CO_2) containing 1 μM propranolol (Sigma). For intracellular recordings, fibre-filled glass micropipettes filled with 3 mM KCl and of 30–50 Mohm resistance were used. Before recordings were made, the tissue was allowed at least 1 h to equilibrate. Recordings were displayed on a Gould OS 1420 oscilloscope and stored on a TEAC cassette recorder.

Student's *t* tests were used for statistical analysis of data, and a *P* value of less than 0.05 was considered statistically significant.

All experiments were carried out in the dark unless otherwise stated. Fibre-optic lights (Dolan-Jenner, illumination of about 3,000 foot candles) were used for illumination in light-on experiments.

Both Bay K 8644 and nifedipine were gifts from Miles Laboratory and were dissolved in ethanol. The final ethanol concentrations were less than 0.01% and had no significant effect on the electrical and mechanical responses of the arteries.

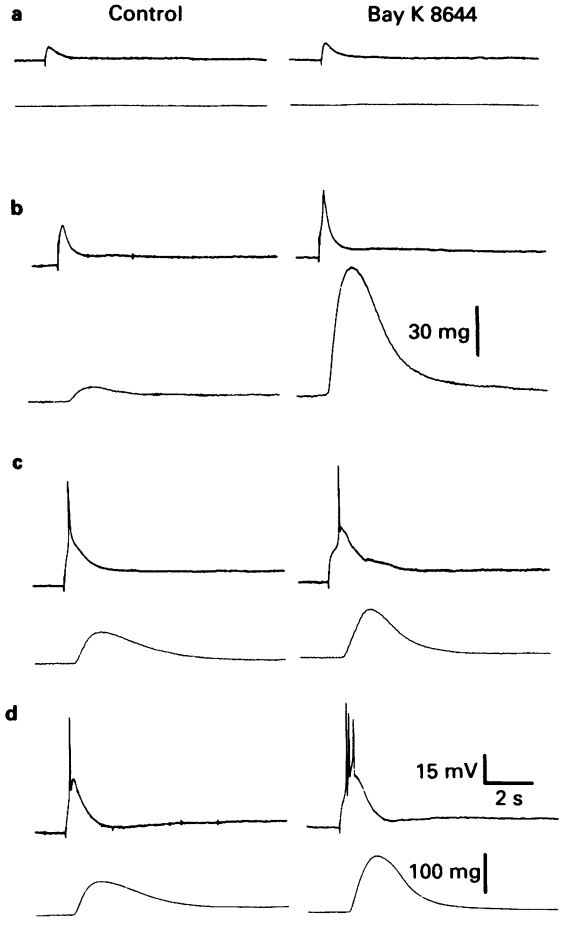

Figure 1 Effect of Bay K 8644 (7×10^{-8} M) on the electrical (top traces) and mechanical (bottom traces) responses of the rabbit ear artery to neural stimulation. (a) Stimulation at 30 V and 0.1 ms duration elicited a subthreshold e.j.p. (left panel). Bay K 8644 had no significant effect on the e.j.p. (right panel). (b) Stimulation at 60 V and 0.1 ms duration elicited a 'peaky' e.j.p (left panel) which developed into an action potential in the presence of Bay K 8644 (right panel). The resulting contraction also increased significantly. (c) In another preparation, similar stimulation at 70 V and 0.1 ms elicited a full size action potential and a strong contraction in the control condition (note change in calibration scale for tension). The potentiating effect of Bay K 8644 (right panel) was less than that produced in (b). (d) In two preparations, three action potentials could be triggered from one e.j.p. in the presence of Bay K 8644.

Results

The membrane of the distal rabbit ear artery was electrically and mechanically quiescent. The resting membrane potential was stable and averaged -65.8 ± 0.6 mV ($n = 30$ preparations). Stimulation of perivascular nerves elicited e.j.ps in the vascular smooth muscle (Figure 1a). If the amplitude of the

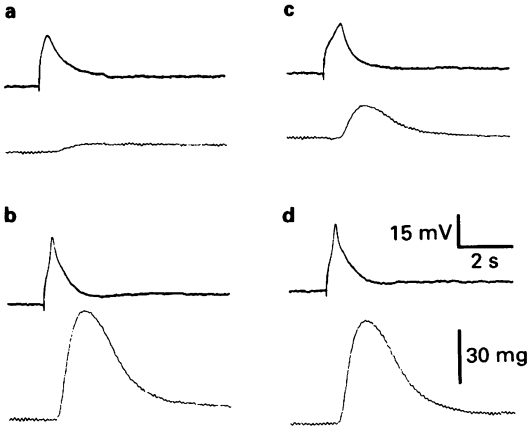

Figure 2 Effect of light on the potentiating effects of Bay K 8644. (a) Under control conditions, stimulation at 60 V and 0.1 ms duration elicited a 'peaky' e.j.p. and a small contraction. (b) In the presence of Bay K 8644 (7×10^{-8} M), both the electrical and mechanical responses were potentiated. (c) With exposure to light for 20 s, the increases in the electrical and mechanical responses were inhibited. (d) The potentiating effect of Bay K 8644 recovered after the light was turned off. All recordings were made in the same cell.

e.j.p. was large enough to reach a threshold potential of about -52.8 ± 1.30 mV ($n = 18$ preparations), a small active response was observed on the top of the e.j.p. (Figure 1b). This type of small active response has been termed a 'peaky' e.j.p., and it is known to be associated with small contractions in arteries (Holman & Surprenant, 1979; Surprenant, 1980). The active response developed into a full action potential if the stimulus intensity was further increased (Figure 1c and d). The resulting contractions were also much larger than those produced from 'peaky' e.j.ps.

At low concentrations of Bay K 8644 (7×10^{-8} M), there was no significant change in resting membrane potential (-64.6 ± 1.2 mV; control = -65.5 ± 1.4 mV; $n = 12$ preparations). Bay K 8644 also had no appreciable effect on the e.j.p. (Figure 1a) but the active responses were potentiated differentially according to the initial response. When the e.j.p. was at the threshold potential, the 'peaky' e.j.p. developed into a fully discernible action potential in the presence of Bay K 8644 (Figure 1b; amplitude = $155 \pm 21\%$ of control). The resulting contraction also increased significantly to $556 \pm 78\%$ of control. If the action potential was already well-developed under the control conditions there was very little further increase (to $107 \pm 3.9\%$ of control) in the amplitude of the action potential (Figure 1c) although, in two of these preparations, multiple action potentials were elicited (Figure 1d). Under control conditions, only one action potential per e.j.p. could be triggered. The contractions also increased to $214 \pm 28\%$ of control.

The effects of Bay K 8644 were inhibited by light. Figure 2 demonstrates these effects as recorded continuously in the same cell. Figure 2a shows an e.j.p. that just reached threshold and the small contraction elicited. In the presence of Bay K 8644 (7×10^{-8} M), the amplitude of both the electrical and mechanical responses were potentiated (b). After exposing the preparation to light for 20 s, the responses were diminished (c). The potentiating effect of Bay K 8644

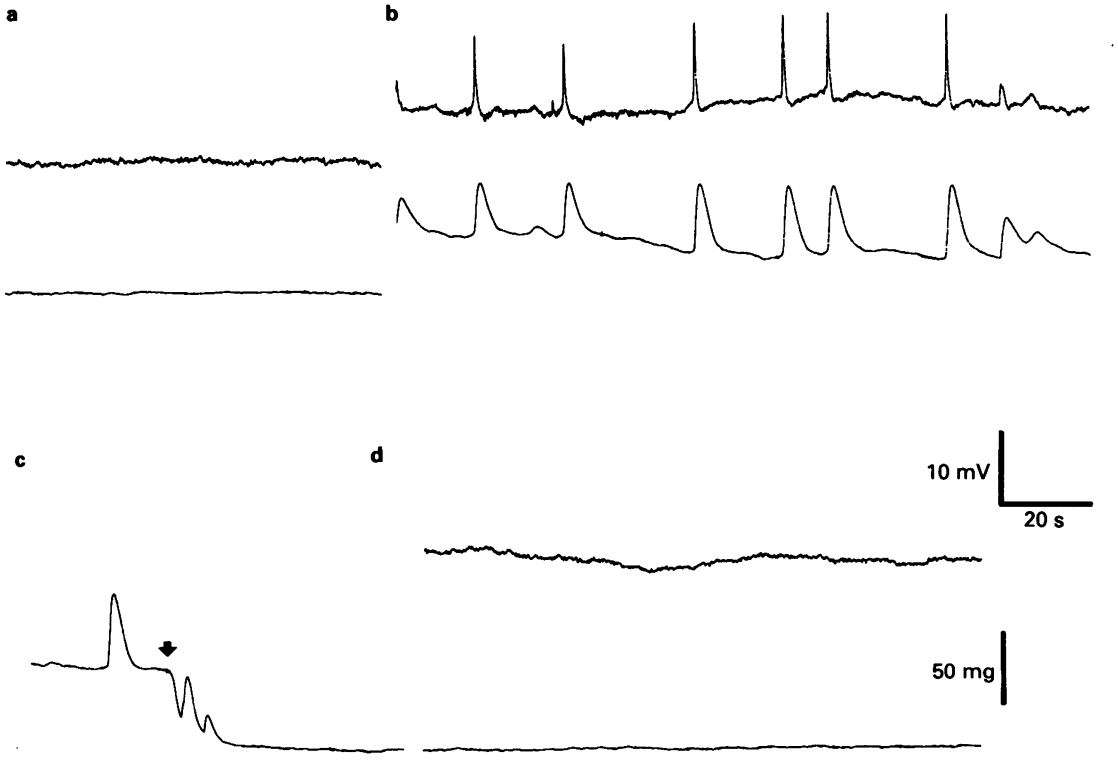

Figure 3 Effect of a high concentration of Bay K 8644 on spontaneous activity of the rabbit ear artery. (a) Control; the rabbit ear artery was electrically and mechanically quiescent. The resting membrane potential was -67 mV. (b) In the presence of Bay K 8644 (3×10^{-6} M), the membrane depolarized to -55 mV and spontaneous tone developed. There were also spontaneous action potential activities accompanied by fast twitch-like contractions. (c) Exposure to light caused a prompt relaxation of the artery. (d) The membrane depolarization and spontaneous activity were abolished in the presence of light.

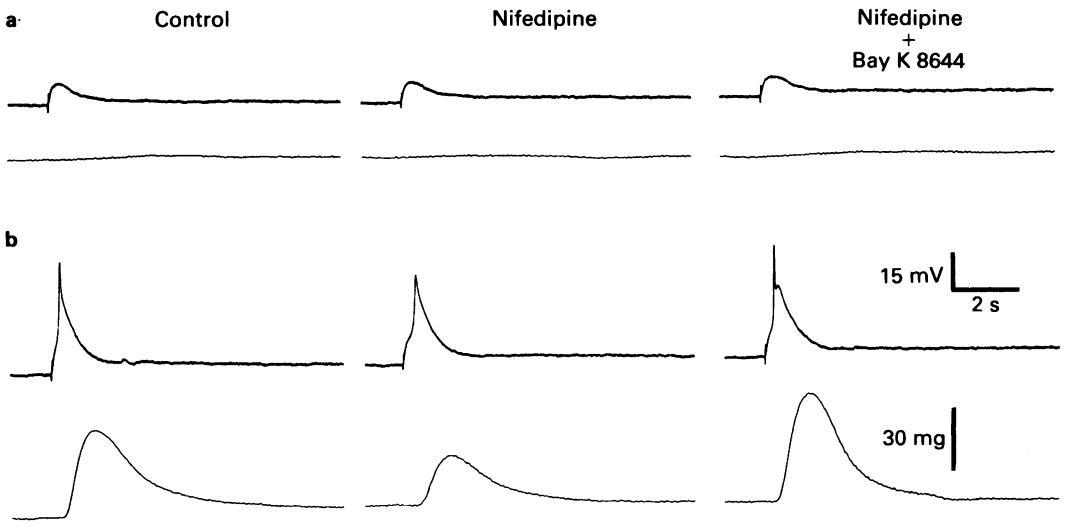

Figure 4 Effect of nifedipine on neural responses of the rabbit ear artery. Nifedipine (3×10^{-7} M) had no significant effect on the amplitude of the e.j.p. (a). However, the amplitude of the action potential and contraction were significantly depressed (b). The effect of nifedipine could be antagonized by Bay K 8644 (right panels). All recordings were made in the same cell.

fully recovered after the light had been turned off for 30 s (d). Under control conditions, the electrical and mechanical responses of the rabbit ear artery were not affected by light.

At high concentrations, Bay K 8644 (3×10^{-6} M) induced spontaneous vascular tone and depolarized the membrane to -51.7 ± 2.1 mV (control = -66.0 ± 1.1 mV, $n = 4$ preparations). Spontaneous action potentials accompanied by fast twitch-like contractions were also observed (Figure 3). These Bay K 8644-induced responses could be reversed by light (Figure 3c and d). Thus the spontaneous action potentials and the accompanying contractions were suppressed while the basal tone and membrane potential reversed to the control level of -65.7 ± 1.2 mV.

Nifedipine (3×30^{-7} M) had no significant effect on the resting membrane potential (-64.4 ± 1.8 mV; control = -64.7 ± 1.6 mV, $n = 6$) and the e.j.p. The fully developed action potential and the resulting contraction, however, were depressed to $82 \pm 1\%$ and $45 \pm 6\%$ respectively (Figure 4). Nifedipine did not alter the threshold potential of the action potentials and its inhibitory action could be reversed by the addition of Bay K 8644 (Figure 4). Unlike Bay K 8644, the inhibitory effect of nifedipine was not readily reversed by exposure to light.

Discussion

Stimulation of perivascular nerves with single pulses elicited e.j.ps in the distal rabbit ear artery. The slow depolarization component present in some blood vessels (Cheung, 1982; 1985a) was not observed. Similar to the tail artery and the saphenous vein of the rat, the e.j.p. itself did not generate any contraction unless a threshold potential was reached (Cheung, 1984, 1985a). Both Bay K 8644 and nifedipine had no significant effect on the e.j.ps.

In cardiac muscle cells, Bay K 8644 increases the current amplitude (Kokubun & Reuter, 1984; Thomas *et al.*, 1985) and the probability of a single calcium channel re-opening during depolarization (Brown *et al.*, 1984), and prolongs the mean opening time of the channel (Kokubun & Reuter, 1984; Hess *et al.*, 1984). As a result, the action potential duration is increased (Brown *et al.*, 1984; Thomas *et al.*, 1985). In the rabbit ear artery, Bay K 8644 increased the amplitude of the action potential. These findings suggest that the calcium agonist promotes calcium entry during the action potential. In control conditions, only one action potential was observed per e.j.p., even at supramaximal stimulation. In the presence of Bay K 8644, multiple action potentials could be trig-

gered from one e.j.p. This is consistent with the finding in cardiac muscle that the probability of calcium channels re-opening, having opened and closed, is enhanced by Bay K 8644 (Brown *et al.*, 1984).

Contraction in vascular smooth muscle mediated by membrane depolarization is enhanced by Bay K 8644 and depressed by nifedipine (Schramm *et al.*, 1983; Cheung, 1985b; Spedding, 1985; Mikkelsen, 1985). The effects of Bay K 8644 and nifedipine on spontaneous contraction could be explained by increases and decreases in calcium influx respectively (Schramm *et al.*, 1985). From studies in skinned vascular smooth muscle, it has been shown that Bay K 8644 has no effect on the contractile proteins and internal calcium storage sites (Kanmura *et al.*, 1984). The Bay K 8644 concentration used in the present study also had no significant effect on transmitter release in the rabbit ear artery (Pan *et al.*, 1985).

An increase in vascular tone with Bay K 8644 that was dependent on extracellular calcium was observed in the rat aorta (Mikkelsen *et al.*, 1985) but not in rabbit aorta (Schramm *et al.*, 1983), the tail artery and the saphenous vein of the rat (Cheung, unpublished observations). We also observed development of vascular tone in the rabbit ear artery with higher concentrations of Bay K 8644. The increase in tone was accompanied by membrane depolarization in the rabbit ear artery. These findings suggest that Bay K 8644 at high concentrations has a direct effect on the calcium channels in the resting state.

The inotropic effect of Bay K 8644 in cardiac muscle is frequency-dependent. No inotropic effect was observed with low frequency stimulation (0.003 Hz) and maximal effect was obtained at 0.5 Hz (Thomas *et al.*, 1985; Kennedy & Seifen, 1985). In the present study, only single stimuli were applied with pulse intervals ranging from 4 to more than 20 min. In all cases, potentiation of the contraction and the action potential were observed, indicating that the effect of Bay K 8644 was not frequency-dependent in the rabbit ear artery.

The calcium antagonist nifedipine, which is very similar in structure to Bay K 8644, suppressed contraction by inhibiting the action potential of the rabbit ear artery. This inhibition was reversed by Bay K 8644. This was in agreement with the proposal that these dihydropyridines act on the same receptor site (Janis & Triggle, 1984; Su *et al.*, 1984; Thomas *et al.*, 1984; Schramm *et al.*, 1985, Spedding, 1985). One very interesting observation from the present study was the difference in sensitivity to light between Bay K 8644 and nifedipine. While the effect of Bay K 8644 was exquisitely sensitive to light, the effect of nifedipine was not changed under similar experimental conditions.

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