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Comparison of effects of a new dihydropyridine, Bay K 8644, and nifedipine on spontaneous mechanical activity in rat portal vein

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1 In isolated portal veins from rats, Bay K 8644 (methyl-1, 4-dihydro-2, 6-dimethyl-3-nitro-4 (2-trifluoromethyl-phenyl) pyridine-5-carboxylate) increased the spontaneous mechanical activity in low but not in high concentrations.

2 The Bay K 8644-induced increase in spontaneous mechanical activity was abolished in Ca-free medium and restored by readdition of Ca.

3 Nifedipine abolished the augmenting effect of Bay K 8644 on the spontaneous mechanical activity; this effect of nifedipine could be eliminated by further increasing the concentration of Bay K 8644.

4 The results are consistant with the conclusion that in rat portal vein, Bay K 8644 increases the entry of extracellular Ca by a mechanism antagonistic to that of nifedipine and in high concentration has a Ca-entry blocking effect.

Introduction

Bay K 8644 (methyl-1, 4-dihydro-2, 6-dimethyl-3-nitro-4 (2-trifluoromethyl-phenyl) pyridine-5-carboxylate) a CF_3 analogue of nifedipine is a member of a new group of dihydropyridines which stimulate the contractile process in heart and vascular smooth muscle (Schramm *et al.*, 1983). Studies on rabbit and rat isolated aorta indicate that Bay K 8644 acts mainly by enhancing the transmembrane influx of calcium and thus, seems to have a mechanism of action opposite to that of nifedipine (Schramm *et al.*, 1983); Mikkelsen *et al.*, 1984b).

Previous studies have shown that the contractile activity in the spontaneously active smooth muscle of the rat portal vein is highly dependent on the extracellular Ca-concentration and that Ca-antagonistic drugs, including nifedipine, have an inhibitory effect on the spontaneous mechanical activity in these vessels (Axelsson *et al.*, 1967; Pegram & Ljung 1981; van Neuten & Vanhoutte, 1981; Mikkelsen *et al.*, 1984). Thus, the rat portal vein is suitable for studies of effects of compounds which act on the transmembrane influx of calcium. Therefore, in order to study the action of Bay K 8644 further, the effect of the dihydropyridine was studied on the spontaneous mechanical activity in rat isolated portal vein and compared to that of nifedipine.

Methods

Male Wistar-Kyoto rats (WKY) (own breed), aged about 4 months were used. The animals were killed by a blow to the neck. The portal veins were removed and cut longitudinally. Preparations of about 4 mm in length were mounted vertically in 30 ml organ baths containing Krebs solution (37°C) and bubbled continuously with a mixture of 95% O_2 and 5% CO_2 to obtain a pH of 7.4. Isometric tension was recorded by a Grass FTO3 transducer connected to a Beckmann polygraph (model R611). The optimal resting tension for recording spontaneous mechanical activity and active tension in the portal vein preparations was 10 mN. When the spontaneous, mechanical activity was stable, the effect of Bay K 8644 nifedipine and Ca-free medium was studied.

Solutions and drugs

A Krebs solution of the following composition (mM) was used: NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.5 and glucose 11. In Ca-free medium $CaCl_2$ was omitted from the Krebs solution and replaced by 0.1 mM EGTA.

Bay K 8644 (Bayer AG), nifedipine (Adalat Bayer

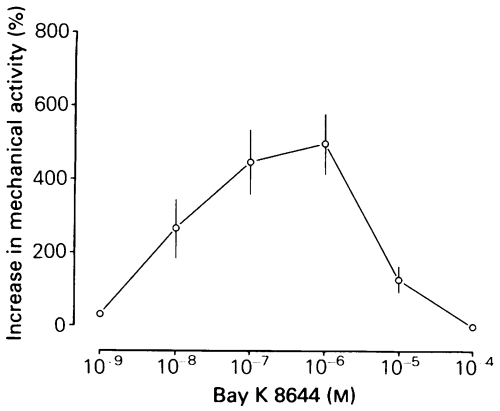

Figure 1 Effect of Bay K 8644 on spontaneous mechanical activity of rat portal vein. Each point is the mean of $n = 5$. Vertical lines show s.e.mean.

AG), Bay K 8644 and nifedipine were dissolved in ethanol 96% and added to the organ baths in volumes of 0.03–0.3 ml.

Results

Bay K 8644 (10^{-9}–10^{-6}M) induced a concentration-dependent increase of the spontaneous mechanical activity in rat portal vein; a further increase in Bay K 8644 concentration depressed the mechanical response towards the control level (Figure 1).

The enhancing effect of Bay K 8644 (10^{-8}M) on the spontaneous activity was eliminated in Ca-free medium while readdition of Ca 1.5 mM restored the spontaneous mechanical activity (Figure 2). Nifedipine had a concentration-dependent inhibitory effect on Bay K 8644-induced increase in spontaneus mechanical activity; nifedipine (10^{-7}M) totally prevented the effect of Bay K 8644 (10^{-8}M). However, the inhibitory effect of nifedipine on the Bay K 8644-induced increase in spontaneous activity was itself eliminated by a further increase in the Bay K 8644 concentration (Figure 2).

Discussion

The findings that the potentiating effect of Bay K 8644 on the spontaneous mechanical activity of rat portal veins could be eliminated effectively in Ca-free medium and re-established by addition of Ca suggest that the effect of Bay K 8644 is dependent on influx of Ca from the extracellular medium; furthermore, it strongly indicates that the compound acts by enhancing Ca-influx. This is also supported by studies of Schramm & Towart (1984) who found that Bay K 8644 increases ^{45}Ca influx into rabbit aortic rings. Previous studies of Schramm *et al.* (1983) indicate that nifedipine has a competitive inhibitory effect on Bay K 8644-induced tone in the rabbit isolated aorta. On the basis of this finding they characterized Bay K 8644 as having an action opposite to that of nifedipine. Previous studies on rat aorta also indicate that Bay K

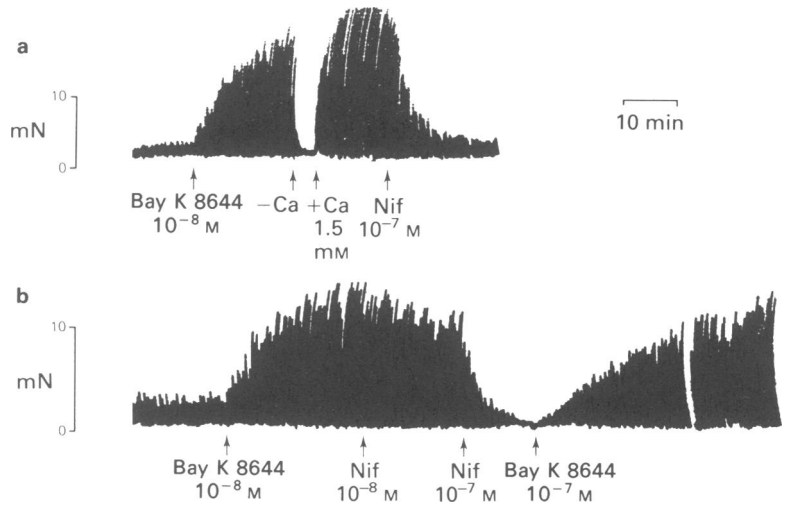

Figure 2 Effect of Bay K 8644 (10^{-8}M) on spontaneous mechanical activity in rat portal veins. (a) Effect of Ca-free medium (− Ca), addition of Ca 1.5 mM (+ Ca) and nifedipine (Nif) 10^{-7}M on Bay K 8644 (10^{-8}M)-enhanced mechanical activity. (b) Inhibitory effect of nifedipine (Nif) (10^{-8}–10^{-7}M) on Bay K 8644 (10^{-8}M)-induced increase in spontaneous activity and the effect of further addition of Bay K 8644 (10^{-7}M).

8644, in contrast to nifedipine, acts mainly by enhancing Ca influx (Mikkelsen) *et al.*, 1985). That Bay K 8644 could stimulate Ca-influx in a manner opposite to that of the Ca-entry blocking effect of nifedipine is also supported by the present results: nifedipine effectively prevented the Ca-dependent Bay K 8644-enhanced mechanical activity in the portal vein which, again, could easily be restored by further addition of Bay K 8644. However, the findings that Bay K 8644 in low concentrations enhanced and in high concentrations depressed the spontaneous contractions reveal a dual effect, which could imply both a Ca-enhancing and Ca-inhibitory mechanism of action, thus indicating that the specificity of Bay K 8644 as a Ca-influx enhancing agent is only relative.

References

AXELSSON, A., WAHLSTRÖM, B., JOHANSSON, B. & JONSSON, O. (1967). Influence of the ionic environment on spontaneous electrical and mechanical activity of the rat portal vein. *Circulation Res.*, **XXI**, 609–618.

MIKKELSEN, E., JESPERSEN, L.T. & LEDERBALLE PEDERSEN, O. (1984). Effects of two new Ca-entry blockers bepridil and nitredipine on isolated vessels. *Acta pharmac. tox.*, **54**, 258–264.

MIKKELSEN, E., NYBORG, N.C.B. & KAZDA, S. (1985). A novel 1,4 dihydropyridine, BAY K 8644, with contractile effect on vascular smooth muscle. *Acta pharmac. tox.*, **56**, 44–49.

PEGRAM, B.L. & LJUNG, B. (1981). Neuroeffector function of isolated portal vein from spontaneously hypertensive and Wistar-Kyoto rats: dependence on external calcium concentration. *Blood Vessels*, **18**, 89–99.

SCHRAMM, M., THOMAS, G., TOWART, R. & FRANCKOWIAK, G. (1983). Activation of calcium channels by novel 1,4-dihydropyridines. A new mechanism for positive inotropics of smooth muscle stimulants. *Arzneim. Forsch. Drug Res.*, **33**, 1268–1272.

SCHRAMM, M. & TOWART, R. (1984). Modulation of calcium channel function with the 1,4-dihydropyridine structure. *J. Physiol.*, (in press).

VAN NUETEN, J.M. & VANHOUTTE, P.M. (1981). Calcium entry blockers and vascular smooth muscle heterogeneity. *Fedn Proc.*, **40**, 2862–2865.

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