Itzhak, Y., Bonnet, K. A., Groth, J., Miller, J. M., Simon, E. J. (1982) Life Sci. 31: 1363–1366

Kosterlitz, H. W., Paterson, S. J., Robson, L. E. (1981) Br. J. Pharmacol. 73: 93ᶜ-969

Kuhar, M. J., Pert, C. Ь Snyder, S. H. (1973) Nature 265: 447–450

Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E., Gilbert, P. E. (1976) J. Pharmacol. Exp. Ther. 197: 517–532

Maurer, R. (1982) Neurosci. Lett. 30: 303–307

McPherson, G. A. (1983) Computer Programs in Biomedicine 17: 107–114

Pasternak, G. W. (1980) Proc. Natl. Acad. Sci. USA 77: 3691–3694

Pfeiffer, A., Herz, A. (1982) Mol. Pharmacol. 21: 266–271

Pfeiffer, A., Pasi, A., Mehraein, P., Herz, A. (1982) Brain Res. 248: 87–96

Spokes, E. G. S., Koch, D. J. (1978) J. Neurochem. 31: 381–383

Su, T. P. (1985) J. Pharmacol. Exp. Ther. 232: 144–148

Syapin, P. J., Ritchie, T., Noble, L., Noble, E. P. (1987) J. Neurochem. in press

Tam, S. W. (1985) Eur. J. Pharmacol. 109: 33–41

Wood, P. L., Charleson, S. (1982) Neuropharmacology 21: 266–271

Wood, P. L., Charleson, S., Lane, D., Hudgin, R. L. (1981) Ibid. 20: 1215–1220

J. Pharm. Pharmacol. 1987, 39: 954–957
Communicated March 16, 1987

The effects of the calcium channel agonist, Bay K-8644, on guinea-pig ileum and rat uterine horn

DIANA CONTE-CAMERINO*, MARCELLO D. LOGRANO, ANNAMARIA DE LUCA, MICHELE PERSICHELLA, FLAVIA FRANCONI†, *Unit of Pharmacology and Pharmacognosy, Department of Pharmacobiology, University of Bari, Via Amendola 165, Bari, †Department of Preclinical and Clinical Pharmacology 'M. A. Mancini' University of Florence, Viale G. B. Morgagni, 65 Florence, Italy*

Bay K-8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate) concentration-dependently caused contractions of the partly depolarized ileum, but at higher concentrations (10^{-6} to 10^{-5} M) produced relaxation. 10^{-8} to 4×10^{-7} M nifedipine antagonized while 10^{-9} and 4×10^{-9} M potentiated Bay K-8644. On the partly depolarized uterus, Bay K-8644 (10^{-9} to 10^{-6} M) had only a spasmogenic effect whereas nifedipine (4×10^{-9} to 10^{-7} M) was spasmolytic. Verapamil and diltiazem (each at 10^{-4} M) both reduced the maximal response to Bay K-8644, while other spasmolytics were ineffective. Thus, Bay K-8644 activates ileum and uterus by opening voltage-operated Ca^{2+} channels, but its relaxant action at high concentration and its potentiation by nifedipine is not seen in both organs. Such differences probably depend on the concentration of the compounds used and the polarization state of the cell membranes.

The dihydropyridine, Bay K-8644, is a Ca^{2+} channel activating drug which promotes transmembrane Ca^{2+} influx through voltage-operated channels (Schramm et al 1983). Its effects are competitively antagonized by nifedipine, while non-dihydropyridine Ca^{2+} antagonists, such as verapamil and diltiazem, exert only functional antagonism (Schramm et al 1983; Ishii et al 1985). Although voltage-operated Ca^{2+} channels play an important role in the potential-dependent contractile responses of all smooth muscles (Hurwitz 1986), only a few studies (Allen et al 1985; Spedding 1985) have been made of Bay K-8644's effects on non-vascular smooth muscles. The purpose of the present study was to evaluate the effects of Bay K-8644 on ileum and uterus.

* Correspondence and present address: Istituto di Farmacologia, Policlinico, Piazza G. Cesare, 70124 Bari, Italy.

Materials and methods

The first 5 cm of the ileum nearest to the ileocaecal valve were dissected from male guinea-pigs (300–350 g) and vertically mounted in a 20 mL organ bath under a tension of 0·5 g. Mechanical activity was recorded isotonically on smoked drums. During the experiments different bathing solutions were used. The normal medium had the following composition (mM): NaCl 119, KCl 4·7, $MgSO_4 \cdot 7H_2O$ 1·5, $CaCl_2$ 2·5, KH_2PO_4 1·18, $NaHCO_3$ 25, glucose 11. An 8 mM KCl-enriched medium was prepared by equimolar substitution of NaCl in the normal medium, whereas an 8 mM K^+-rich, Ca^{2+}-free medium was obtained from the K^+-enriched medium by omitting $CaCl_2$ and adding the equimolar amount of Cl^- as NaCl and 0·1 mM of EGTA. The bathing fluids were gassed with a mixture of 5% CO_2 and 95% O_2 to give a pH of 7·3–7·4, and were maintained at 37 °C.

After 60 min equilibration in normal medium, the preparations were repeatedly exposed to 50 mM KCl until the contractile responses were reproducible, and these were defined as 100% control responses. Afterwards the effect of Bay K-8644 was tested on different groups of tissues in the following experimental conditions: (i) in preparations perfused in normal medium; (ii) in preparations partly depolarized by perfusion with 8 mM K^+-enriched medium (subthreshold concentration for contraction) for 30 min; (iii) in preparations perfused with 8 mM K^+-rich, Ca^{2+}-free medium for 30 min. Bay K-8644 was added to the muscle bath in a

cumulative fashion; the interval between successive additions of the agonist was adjusted to allow sufficient time for the response to develop fully.

The effects of the drugs used to counteract the action of Bay K-8644 were evaluated separately, on partly depolarized tissues (group ii) as follows: (1) nifedipine was added 30 min before Bay K-8644 and its effect was tested against the whole cumulative concentration-response curve to Bay K-8644; (2) diltiazem and verapamil, atropine, papaverine and dantrolene sodium were tested separately on different tissues maximally contracted by Bay K-8644. Tetrodotoxin preincubated 10 min before the agonist was tested against the whole cumulative concentration-response curves of Bay K-8644.

Virgin female rats (200–250 g) were ovariectomized under ether anaesthesia. Ten days later they were treated with 0·1 mg kg^{-1} oestradiol benzoate subcutaneously for 3 days. Three hours after the last injection, the rats were killed and uterine horns were removed and vertically mounted in an organ bath under a tension of 0·5 g (Conte-Camerino et al 1983). The contractions were recorded isotonically on smoked drums. After 60 min equilibration in normal medium the preparations were almost maximally activated with oxytocin (5×10^{-7} mM) until reproducible responses were obtained (used as 100% control response).

The experimental protocol followed was that used for ileum except that 9 mM KCl was used to create the depolarizing medium. Drugs used to counteract the action of Bay K-8644 on the uterus included nifedipine and diltiazem, verapamil, atropine, dantrolene sodium, indomethacin, ketoprofen, salbutamol and propranolol.

All the experiments were carried out under sodium lamp light to avoid light-induced oxidation of the dihydropyridines. Bay K-8644 and nifedipine were dissolved in 96% ethanol before dilution in 8 or 9 mM KCl buffer. Ethanol at its final concentration, had no effect on the contractions induced by 50 mM KCl and oxytocin. All values are mean ± s.e.m. A paired or unpaired Student's *t*-test was used to evaluate the statistical significance of the effects. The criterion for statistical significance was $P < 0.05$. Drugs used included: Bay K-8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxylate, Bayer); nifedipine (Bayer); verapamil hydrochloride (Knoll); diltiazem hydrochloride (Sigma Tau); atropine sulphate (Sigma); tetrodotoxin (Sankyo). Other chemicals were of analytical grade and were purchased from commercial sources.

Results

In normal medium or in a K^+-rich, Ca^{2+}-free medium Bay K-8644 was not effective in evoking spasm either of the ileum or of the uterus. But on partly depolarized ileum, Bay K-8644 concentration-dependently increased the tension (−log EC50 7·8 ± 0·03) and spontaneous motility (Figs 1A and 2A). As shown in Fig. 1A at the highest concentrations used (>10^{-6} M), Bay K-8644 produced a concentration-dependent decrease of its own contraction. At concentrations less than 10^{-6} M the effect of Bay K-8644 showed no tendency to fade during 6–8 h and was not reversible by washing. In the presence of 1×10^{-8} to 4×10^{-7} M nifedipine the concentration-response curve to Bay K-8644 moved in a parallel way to the right (Figs 1B, 2B). Low nifedipine concentrations (1×10^{-9} and 4×10^{-9} M) shifted the concentration-response curve of Bay K-8644 to the left (Figs 1B, 2C). In this respect the spasmogenic effect of low Bay K-8644 concentration was enhanced.

Diltiazem and verapamil, separately, in a concentration range between 10^{-8} to 6×10^{-5} M, failed to inhibit significantly the spasmogenic effect of Bay K-8644. On

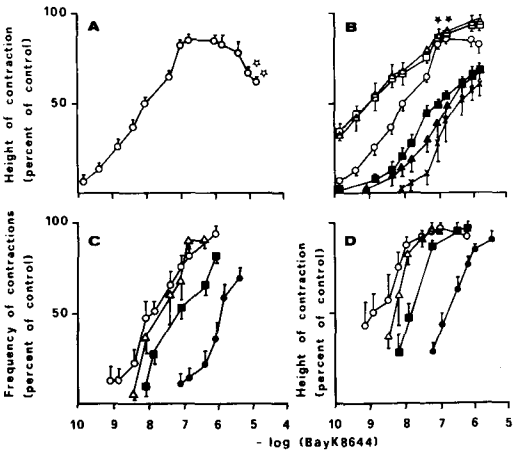

FIG. 1. Upper panel: concentration-response curves of the effect of Bay K-8644 on partly depolarized guinea-pig ileum in absence (A) and presence (B) of increasing concentrations of nifedipine. Contractions are expressed as percentage of the response to 50 mM KCl (defined as 100%). Each point represents the mean response ± s.e.m. of 9 (A) and 6–9 (B) experiments. The ○ nifedipine curve (-○-) in B is taken from A. Note (A) that at the highest concentrations Bay K-8644 causes inhibition of its own contraction (*$P <$ 0·001) and (B) that nifedipine causes a parallel shift to the right, but at the lower doses it potentiates Bay K-8644. All points (except those with asterisk *) of the nifedipine curves are significantly different ($P < 0.05$ or less) from the corresponding values of Bay K-8644 (○ nifedipine (-○-)) curve. Lower panel: concentration-response curves of the effect of Bay K-8644 on (C) frequency and (D) height of contraction of partly depolarized rat uterine muscle in the absence and presence of increasing concentrations of nifedipine. Frequency and height of contraction are expressed as percentage of the same parameters elicited by 5×10^{-7} M oxytocin (defined as 100%). Each point represents the mean response ± s.e.m. of 5–10 experiments. All points of the nifedipine curves (except 4×10^{-9} M curve), are significantly different from the corresponding values of Bay K-8644 (○ nifedipine) curve. For upper and lower panels nifedipine concentrations (M) are as follows: (-□-) 1×10^{-9}; (-△-) 4×10^{-9}; (-■-) 1×10^{-8}; (-▲-) 8×10^{-8}; (-●-) 1×10^{-7}; (-×-) 4×10^{-7}.

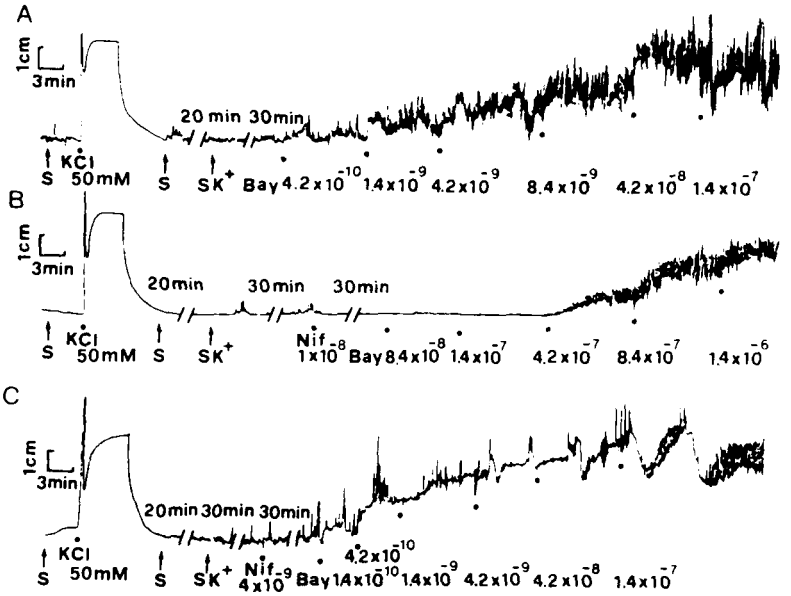

FIG. 2. Traces showing the effect of Bay K-8644 (Bay) on guinea-pig isolated ileum. After an equilibration period of 60 min in normal solution (S), the preparations were near maximally contracted by 5–6 min exposition to 50 mM KCl (control contraction); after stabilization, the tissues were partly depolarized by a perfusion with 8 mM KCl (SK^+). Then, Bay K-8644 was added to the muscle bath in a cumulative fashion, (A) before and (B, C) after the addition of nifedipine (Nif). The contractions of ileum were recorded isotonically. Bay and Nif concentrations are M. The panels are from different experiments. Note that in (B) Nif antagonized, while in (C) Nif potentiated Bay K-8644.

the other hand 10^{-4} M diltiazem and verapamil decreased the maximum ileal contractions induced by Bay K-8644 from 85·7 ± 1·2% (n = 9) to 38·0 ± 9·2% (n = 4) and 41·0 ± 1·1% (n = 4), respectively. Tetrodotoxin (10^{-7} to 10^{-6} M), atropine (10^{-8} to 10^{-4} M), papaverine (10^{-8} to 10^{-4} M) and dantrolene sodium (10^{-7} to 4 × 10^{-5} M) did not show any effect on the ileum maximally contracted by Bay K-8644.

On partly depolarized uterine horns, Bay K-8644 (1 × 10^{-9} to 1 × 10^{-6} M) concentration-dependently increased the frequency and height of contractions (Figs 1C, D, 3A) but with different EC50 values (−log EC50 7·8 ± 0·04 and 8·8 ± 0·03). Bay K-8644 was antagonized by nifedipine (4 × 10^{-9} to 1 × 10^{-7} M) (Fig. 3B); at all concentrations studied nifedipine shifted the Bay K-8644 cumulative concentration-response curves to the right in parallel (Fig. 1C, D).

As in the ileum, in the uterus only the highest concentration of verapamil (10^{-4} M) and diltiazem (10^{-4} M) were able to decrease the height of the Bay K-8644 maximally induced contractions from 95·9 ± 2·4% (n = 10) to 40.0 ± 9·0% (n = 3) and 40·0 ± 1·2% (n = 3), respectively, and the maximal increase in frequency from 81·3 ± 4·5 to 25·1 ± 6·3% and 25·3 ± 8·0%, respectively. 10^{-4} M atropine, indomethacin, ketoprofen and 4 × 10^{-5} M dantrolene sodium each failed to antagonize Bay K-8644. Salbutamol, 2·8 × 10^{-7} M abolished the responses of uterine horns to Bay K-8644 (n = 4). Its inhibitory effect was completely blocked by 1·3 × 10^{-6} M propranolol.

Discussion

Bay K-8644 induced contractions in partly depolarized ileum and uterus as already described in trachea (Allen et al 1985) and in vascular smooth muscle (Schramm et al 1983; Dubé et al 1985). The fact that it had no effect in a Ca^{2+}-free solution, and that partial depolarization by elevation of the external potassium concentration was necessary for an effect, supports the hypothesis that Bay K-8644 acts by enhancing the transmembrane Ca^{2+} influx through voltage-dependent channels. This is corroborated by the competitive antagonism of nifedipine in both models, and by the finding that compounds acting by other mechanisms, like dantrolene sodium which mobilizes intracellular Ca^{2+} pools (Conte Camerino et al 1983), did not modify the action of Bay K-8644. Also, in both models the effects of Bay K-8644 was not mediated by activation of muscarinic receptors, nor by prostaglandins.

In the ileum, the contractile effect of Bay K-8644 declined at high concentrations (>10^{-6} M). Such a reversal of the Bay K-8644 action was also found recently in heart muscle and coronary vasculature (Thomas et al 1984), as well as in aortic smooth muscle (Mikkelsen et al 1985) and in pancreatic beta-cells (Malaisse-Lagae et al 1984). It indicates that the ability

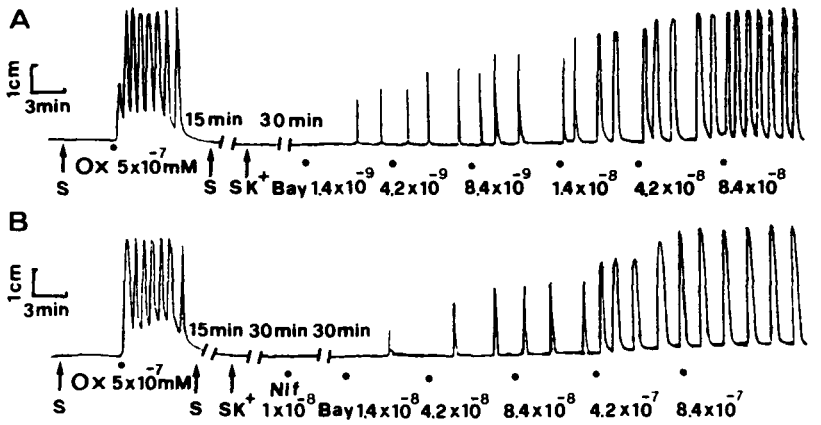

FIG. 3. Traces showing the effect of Bay K-8644 (Bay) on rat isolated uterus. After an equilibration period of 60 min in normal solution (S) the preparations were near-maximally activated with 5×10^{-7} mM oxytocin (Ox, control response). After stabilization the uterus was partly depolarized by exposure in K^+-rich medium (SK^+). Then, Bay was added to the muscle bath in a cumulative fashion, (A) before and (B) after the addition of nifedipine (Nif). The uterine contractions were recorded isotonically. Bay and Nif concentrations are M. The panels are from different experiments.

to inhibit or promote calcium influx may reside in the same molecule (Thomas et al 1984). Though it was shown recently that the (+)-enantiomer of the racemic Bay K-8644 had the properties of a weak Ca-antagonist (Franckowiak et al 1985), it is improbable that the reversal of action in the ileum was due to an overlap of the opposite actions of the two optical isomers since the pure (−)-isomer also exhibits this biphasic behaviour (Thomas et al 1985). This is stressed by the finding that the optically inactive calcium antagonist, nifedipine at low concentrations seemed to enhance the Ca-agonistic action of Bay K-8644 on the ileum by shifting its concentration, response curve to the left and its threshold concentration to lower values. Indeed, it was shown in isolated heart preparations that nifedipine, as well as other dihydropyridine calcium antagonists at low concentration, behaves as a calcium-agonist (Himori et al 1976; Thomas et al 1984). A small potentiation by nifedipine of Bay K-8644-induced tension was also described by Schramm et al (1983) in rabbit aorta. Moreover a potentiating effect of nimodipine on Bay K-8644-induced contractions was described by Dubé et al (1985) in pig coronary artery. That the dual behaviour of the dihydropyridines was not observed on the uterine preparation might be due to our not having used high enough Bay K-8644 concentrations (the highest concentration was $<10^{-6}$ M), or to a different mean membrane potential, since the prevalence of an agonistic or an antagonistic action seems to depend also upon the membrane potential (Sanguinetti & Kass 1984).

The authors wish to thank Dr Günter Thomas from Bayer-Italia, for his helpful suggestions and correction of the manuscript. This work was supported by Italian CNR 85-426 and MPI-85 grants.

REFERENCES

Allen, S. L., Foster, R. W., Small, R. C., Towart, R. (1985) Br. J. Pharmacol. 86: 171–180

Conte-Camerino, D., Lograno, M. D., Siro-Brigiani, G., Megna, G. (1983) Eur. J. Pharmacol. 92: 291–294

Dubé, G. B., Bayk, Y., Schwartz, A. (1985) J. Cardiovasc. Pharmacol. 7: 377–389

Franckowiak, G., Bechem, M., Schramm, M., Thomas, G. (1985) Eur. J. Pharmacol. 114: 223–226

Himori, N., Ono, H., Taira, N. (1976) Jap. J. Pharmacol. 26: 427–435

Hurwitz, L. (1986) Ann. Rev. Pharmacol. Toxicol. 26: 225–258

Ishii, K., Taira, N., Yanagisawa, T. (1985) Br. J. Pharmacol. 84: 577–584

Malaisse-Lagae, N., Mathias, P. C., Malaisse, W. J. (1984) Biochem. Biophys. Res. Commun. 123: 1062–1068

Mikkelsen, E., Nyburg, N. C. B., Kazda, S. (1985) Acta Pharmacol. Toxicol. 56: 44–49

Sanguinetti, M. C., Kass, R. S. (1984) J. Mol. Cell. Cardiol. 16: 667–670

Schramm, M., Thomas, G., Towart, R., Franckowiak, G. (1983) Nature 303: 535–537

Spedding, M. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 328: 464–466

Thomas, G., Gross, R., Schramm, M. (1984) J. Cardiovasc. Pharmacol. 6: 1170–1176

Thomas, G., Franckowiak, G., Bechem, M., Schramm, M. (1985) J. Mol. Cell. Cardiol. 17 (suppl. 3): 168–172