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# THE EFFECTS OF BAY-K-8644 ON THE CONTRACTION OF CAT MIDDLE CEREBRAL AND FEMORAL ARTERIES

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**Abstract**—The effects of BAY-K-8644 on the reactivity of cylindrical segments of cat middle cerebral and femoral arteries were studied. BAY-K-8644 induced dose-dependent contractile responses in cerebral arteries up to $10^{-6}$ M; higher concentrations tended to cause relaxation of the segments. The dihydropyridine elicited contractions in femoral arteries only when these vessels were previously exposed to 15 mM $K^+$. Nifedipine ($3 \times 10^{-7}$ M) produced a parallel shift to the right of the dose–response curve to BAY-K-8644, whereas $5 \times 10^{-6}$ M verapamil markedly reduced the responses evoked by all concentrations of this drug. The removal of $Ca^{2+}$ from the medium abolished the response evoked by the $Ca^{2+}$-channel activator at $10^{-7}$ M in both kinds of arteries. Under these conditions $Ca^{2+}$ addition induced vasoconstriction, which was blocked by nifedipine ($3 \times 10^{-7}$ M). Preincubation of femoral arteries with $10^{-7}$ M BAY-K-8644 potentiated the effects evoked by 25, 50, 75 and 125 mM $K^+$, but did not modify those produced by $10^{-5}$ M noradrenaline. Nifedipine ($10^{-7}$ M and $3 \times 10^{-7}$ M) blocked the potentiation caused by this drug in a dose-dependent manner. Both the increase of the response elicited by BAY-K-8644 and the inhibitory effects of nifedipine were greater at 25 mM $K^+$ than at 125 mM. These results suggest that (1) BAY-K-8644 facilitates $Ca^{2+}$ influx into smooth muscle through $Ca^{2+}$ channels that are possibly voltage sensitive and (2) the voltage independence of the drug-induced contractions in cerebral arteries.

The contraction of smooth muscle is produced by an increase in the cytosolic-free $Ca^{2+}$ [1, 2]. This increase may be caused by an influx of $Ca^{2+}$ from the extracellular medium through slow $Ca^{2+}$ channels [2] whose characteristics have been studied in several tissues using $Ca^{2+}$-channel-blocking agents of the dihydropyridine type, e.g. nitrendipine and nifedipine [3]. Recently minor changes in the molecule of nifedipine have produced BAY-K-8644, which has opposite effects to $Ca^{2+}$ antagonists [4]. Thus whereas these antagonists elicited vasodilatation due to a transmembrane blockade of $Ca^{2+}$ influx [2, 5], BAY-K-8644 promotes $Ca^{2+}$ entry into peripheral smooth and cardiac muscle [4] and adrenal glands [6, 7], inducing contraction and an increase in catecholamines release, respectively. This agent therefore seems to behave as a $Ca^{2+}$-channel activator [4, 6, 7]. These experiments on BAY-K-8644 were performed in peripheral tissues. Since the cerebral vessels are sensitive to $Ca^{2+}$ antagonists producing vasodilation [8–10], one can assume that a $Ca^{2+}$-channel activator such as BAY-K-8644 will induce a contractile response in the vascular bed by increasing $Ca^{2+}$ influx. For this reason in the present study we have compared the effect of the drug on isolated cat middle cerebral and femoral arteries.

## MATERIALS AND METHODS

Cats of either sex (1.5–4 kg) were anaesthetized with sodium pentobarbitone and killed by bleeding. Femoral arteries and the brain were carefully removed and middle cerebral arteries were dissected. Both kinds of vessels were cut into cylindrical segments of 4 mm in length. Each cylinder was set up in an organ bath, as described by Nielsen and Owman [11], containing 6 ml of Krebs–Henseleit solution (KHS) at 37° bubbled continuously with a mixture of $O_2/CO_2$ (95%:5%), pH 7.4. Two stainless-steel pins, 150 $\mu$m diameter, were introduced through the lumen of each arterial segment. One pin was fixed to the organ-bath wall, while the other was connected to a strain gauge for isometric tension recording. The latter pin was parallel with the former and movable, thus permitting the application of resting tension in a perpendicular plane to the long axis of the vascular cylinder.

The recording system included a force-displacement transducer (Grass FTO3C) connected to a polygraph (Grass Model 7D). A resting tension of 0.5 and 1 g (optimal resting tone) was applied to cerebral and femoral arteries, respectively, which are readjusted every 15 min during the 90-min equilibration period.

The vessels were exposed at the beginning of the experiment to 75 mM $K^+$ to check the functional integrity of the arteries. Thereafter the bath medium was changed several times until the resting tone was recovered. The cumulative dose–response curve to BAY-K-8644 was then carried out. Since BAY-K-8644 did not disappear from the tissue after washing, the arteries submitted to this agent were used once.

The effect of BAY-K-8644 ($10^{-7}$ M) on the contraction induced by different concentrations of $K^+$

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and noradrenaline (NA, $10^{-5}$ M) was analysed by adding the compound to the bath 10 min prior to the administration of vasoconstrictor agents. These experiments were carried out in femoral arteries only, because in cerebral ones BAY-K-8644 had an important vasoconstrictor effect in basal conditions.

When nifedipine ($10^{-7}$–$10^{-6}$ M) or verapamil ($10^{-6}$ and $5 \times 10^{-6}$ M) were used, both drugs were added to the bath 10 min prior to the addition of BAY-K-8644 and remained present throughout the experiment.

In other experiments the arterial segments were exposed for 30 min to $Ca^{2+}$-free medium and immediately later, BAY-K-8644 ($10^{-7}$ M) was added and the cumulative dose–response curve to $CaCl_2$ was determined with or without nifedipine ($3 \times 10^{-7}$ M, 10 min preincubation). The effect of $Ca^{2+}$ suppression and $Ca^{2+}$ addition in femoral arteries partly depolarized with $K^+$ (15 mM) was also analysed.

*Solutions, drugs and statistical evaluation.* The composition of the KHS was (mM): NaCl, 115; KCl, 4.6; $CaCl_2$, 2.5; $KH_2PO_4$, 1.2; $NaHCO_3$, 25; $MgSO_4.7H_2O$, 1.2; glucose, 11.1; disodium salt of ethylenediamine tetraacetic acid ($Na_2EDTA$), 0.003. In high-potassium solution, the NaCl concentration was appropriately reduced in order to maintain isotonicity. $Ca^{2+}$-free KHS was prepared by omitting $CaCl_2$ and $Na_2EDTA$ and 1 mM ethyleneglycol-bis(β-aminoethyl-ether)*N*:*N*′-tetraacetic acid (EGTA) was added. These solutions were freshly prepared.

Stock solutions of BAY-K-8644 ($10^{-2}$ M), nifedipine ($10^{-2}$ M) and verapamil ($10^{-2}$ M) were prepared in 99.5% ethanol and kept at −20°. They were diluted to the concentrations used with KHS.

The drugs used were: noradrenaline bitartrate (Sigma Chemical Co., St. Louis, MO), BAY-K-8644 [methyl-1:4-dihydro-2:6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate] (Bayer, Leverkusen, F.R.G.), nifedipine (Bayer, Leverkusen, A.G.) and verapamil hydrochloride (Knoll A.G.). Statistical significance was evaluated by the Student's *t*-test for independent experiments and P values of 0.05 or less were considered significant.

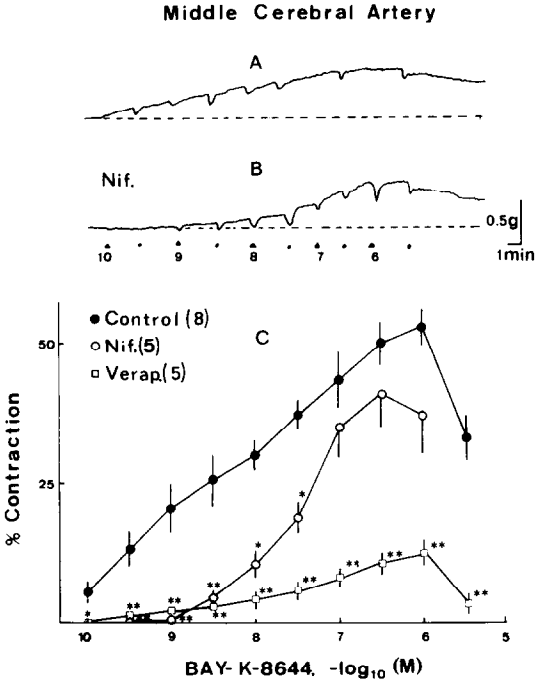


Fig. 1. A recording showing the dose-dependent contraction elicited by BAY-K-8644 in cylindrical segments of cat middle cerebral arteries in resting conditions in the absence (A) and in the presence (B) of nifedipine ($3 \times 10^{-7}$ M, Nif.). (A) and (B) were obtained in different segments. (C) Dose-response curve for BAY-K-8644 in presence or absence of nifedipine ($3 \times 10^{-7}$ M) or verapamil ($5 \times 10^{-6}$ M, Verap.). Responses to BAY-K-8644 were expressed as percentages of the previous contraction induced by 75 mM $K^+$. Isometric responses for 75 mM $K^+$ were of 1371 ± 236 mg. Values are means ± S.E.M. Number of segments used are shown in parentheses (*P < 0.05, **P < 0.005).

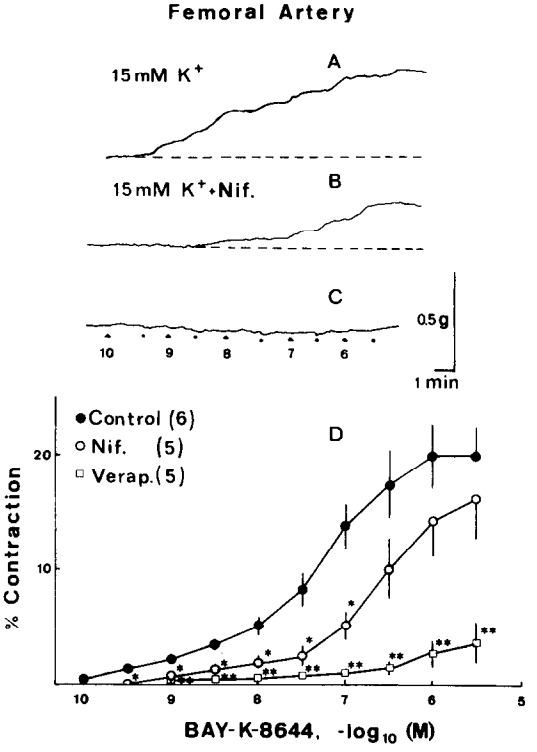


Fig. 2. (A) A recording showing the dose-dependent contraction elicited by BAY-K-8644 in a cylindrical segment of femoral artery partly depolarized with 15 mM $K^+$. (B) Reduction of the contraction by nifedipine ($3 \times 10^{-7}$ M, Nif.). (C) The effect of BAY-K-8644 in a segment without pre-exposure to 15 mM $K^+$. (A), (B) and (C) were obtained in different segments. (D) Dose–response curve for BAY-K-8644 in presence or absence of nifedipine ($3 \times 10^{-7}$ M) or verapamil ($5 \times 10^{-6}$ M, Verap.). Responses to BAY-K-8644 were expressed as percentages of the previous contraction induced by 75 mM $K^+$. Isometric responses for 75 mM $K^+$ were of 2750 ± 225 mg. Values are means ± S.E.M. Number of segments used are in parentheses (*P < 0.05, **P < 0.005).

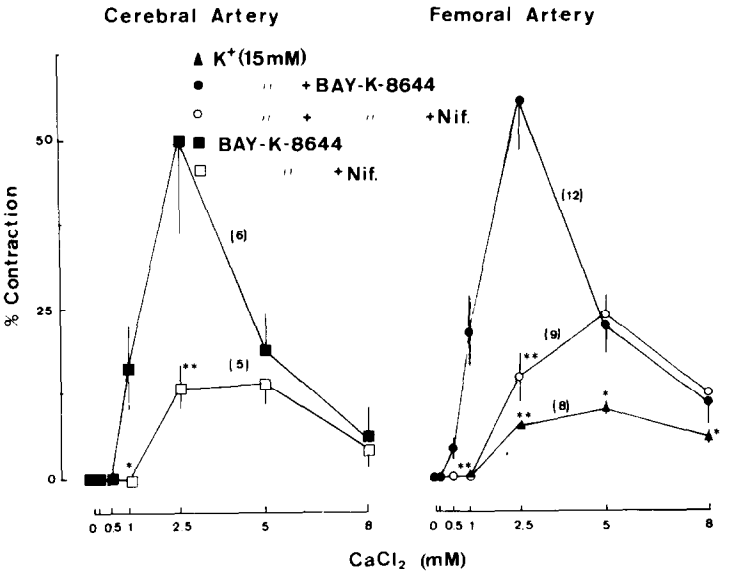


Fig. 3. Effect of $Ca^{2+}$ suppression and addition on the contractions evoked by BAY-K-8644 ($10^{-7}$ M) and BAY-K-8644 plus nifedipine ($3 \times 10^{-7}$ M) in cat middle cerebral arteries and femoral ones partly depolarized with $K^+$ (15 mM). After 30 min exposure to $Ca^{2+}$-free medium, BAY-K-8644 (with or without $K^+$) and thereafter cumulative doses of $Ca^{2+}$ were added to the bath. When nifedipine was used it was administered 10 min before BAY-K-8644. Contractions reached with each $Ca^{2+}$ concentration were expressed as percentages of the contraction induced by 75 mM $K^+$ in normal KHS. Values are means ± S.E.M. Isometric responses for 75 mM $K^+$ were of 1220 ± 190 and 2800 ± 211 mg in cerebral and femoral arteries, respectively. Number of segments used are in parentheses (*$P < 0.05$, **$P < 0.005$).

## RESULTS

BAY-K-8644 ($10^{-10}$–$10^{-6}$ M) elicited dose-dependent vasoconstrictor responses in cat cerebral arteries (Fig. 1); higher concentrations tended to relax the arteries. However, this drug caused a slight contraction in three out of ten femoral arteries. When these vessels were previously exposed to 15 mM $K^+$, BAY-K-8644 ($3 \times 10^{-10}$–$3 \times 10^{-6}$ M) induced vasoconstriction (Fig. 2).

Nifedipine ($3 \times 10^{-7}$ M) shifted the dose–response curve in parallel to the right for BAY-K-8644 using either type of arteries (Figs 1 and 2), whereas at $10^{-6}$ M the vasoconstrictor responses to the $Ca^{2+}$ channel activator were abolished (results not shown). Verapamil ($5 \times 10^{-6}$ M) greatly decreased the contractions evoked by all concentrations of BAY-K-8644 used (Figs 1 and 2), while these were unaffected at $10^{-6}$ M.

$Ca^{2+}$ removal from the extracellular medium blocked the contraction evoked by $10^{-7}$ M BAY-K-8644 in cerebral arteries as well as that elicited in femoral ones previously exposed to 15 mM $K^+$. The subsequent $Ca^{2+}$ addition (0.1–8 mM) to the bath induced vasoconstrictor effects that normally began at 1 mM and reached a maximal response at 2.5 mM; higher concentrations caused relaxation (Fig. 3). When nifedipine ($3 \times 10^{-7}$ M) was present these contractile responses were greatly reduced in cerebral and femoral arteries until 5 mM $Ca^{2+}$ was reached. $Ca^{2+}$ removal also abolished the small contractions induced by 15 mM $K^+$ in KHS in some femoral arteries; the $Ca^{2+}$ addition induced vasoconstriction that began at 2.5 mM and was maximal at 5 mM. Above this concentration the vessels tended to vasodilate (Fig. 3). The contractions induced by $Ca^{2+}$ administration to femoral arteries partly depolarized with $K^+$ (15 mM) were markedly less than those obtained with $K^+$ plus BAY-K-8644 ($10^{-7}$ M).

Preincubation for 10 min with $10^{-7}$ M BAY-K-8644 caused a potentiation of the contractions evoked by 25, 50, 75 and 125 mM $K^+$ in femoral arteries (Fig. 4). This potentiation was decreased when the $K^+$ concentration was increased. Nifedipine ($10^{-7}$ and $3 \times 10^{-7}$ M) inhibited drug-evoked potentiation in a dose-dependent manner. Thus, $10^{-7}$ M nifedipine practically abolished the increase in the response caused by BAY-K-8644 at all concentrations used, although the remaining contraction was similar to that obtained with $K^+$ in absence of both dihydropyridines. At $3 \times 10^{-7}$ M the $Ca^{2+}$ antagonist not only blocked potentiation, but even the residual contraction was significantly reduced with respect to controls. The vasodepressor effects of this concentration of nifedipine was diminished when $K^+$ concentrations were increased, in such a way that at 125 mM $K^+$ this residual contraction was the same as that obtained in controls (Fig. 4). NA ($10^{-5}$ M) induced contractile responses whose magnitude (3052 ± 257 mg) in the presence of $10^{-7}$ or $10^{-6}$ M BAY-K-8644 was of 2888 ± 283 or 2913 ± 216 mg, respectively, i.e. was not significantly modified by the drug.

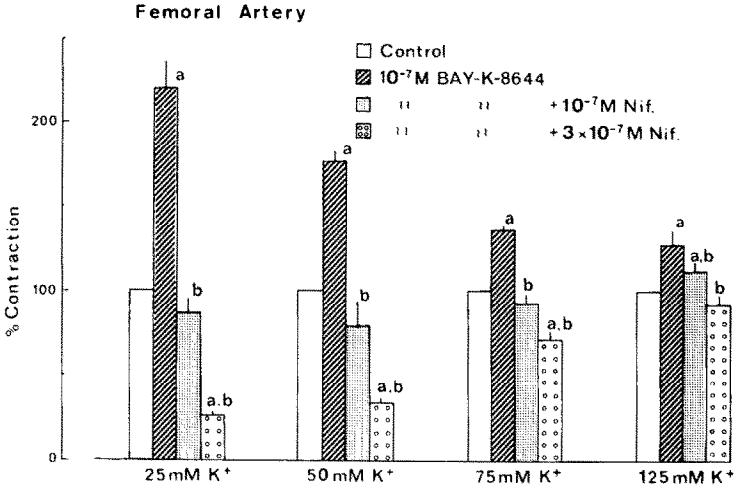


Fig. 4. Potentiation of the contractions caused by different $K^+$ concentrations in cylindrical segments of cat femoral arteries by BAY-K-8644 and the effect on it of nifedipine (Nif.). Results are expressed as percentages of the responses obtained in each control situation. Isometric responses for 25, 50, 75 and 125 mM $K^+$ were of 1262 ± 137, 2502 ± 190, 2883 ± 211 and 2979 ± 236 mg, respectively. Values are means ± S.E.M. Ten to fourteen different arterial segments were used in each case. a indicates statistical significance respect to control $P < 0.05$ and b, statistical significance with regard to the response in the presence of BAY-K-8644, $P < 0.05$.

## DISCUSSION

In the present experiments BAY-K-8644 induced dose-dependent vasoconstrictions in femoral arteries only when they were partly depolarized with 15 mM $K^+$. Since this ion induces direct depolarization of the smooth muscle cells and opens the voltage-sensitive $Ca^{2+}$ channels, increasing the level of intracellular free $Ca^{2+}$ [2, 12], this may indicate that these channels are inactivated under resting conditions in femoral arteries and need to be preactivated by $K^+$. With BAY-K-8644 the necessity for moderate depolarization was also observed in rabbit aortic strips [4] and cat adrenal glands [6], in order to obtain contraction or increased catecholamine release, respectively.

In contrast, in cerebral arteries BAY-K-8644 induced dose-dependent contractile responses under basal conditions, which suggest that in these vessels the voltage-sensitive $Ca^{2+}$ channels might normally be activated or are directly so by the drug. The fact that BAY-K-8644 increases $^{45}Ca^{2+}$ influx in quiescent, isolated, adrenal chromaffin cells slightly but significantly [6] is in agreement with the second hypothesis.

The $Ca^{2+}$ antagonist nifedipine produced a parallel shift to the right of the dose–response curve to BAY-K-8644 in both kinds of arteries suggesting the existence of a competitive antagonism, whereas verapamil produced a non-competitive one, as occurs in rabbit aortic strips [4]. Since the existence of specific dihydropyridine receptors near the slow $Ca^{2+}$ channels has been suggested [6, 13–17], it can be assumed that both dihydropyridines are competing for the same receptors [16, 17]. The interaction of nifedipine with the dihydropyridines will decrease $Ca^{2+}$ entry, and while binding with BAY-K-8644, will increase $Ca^{2+}$ influx as has been suggested [4, 18, 19]. Recently it was reported that the drug prolongs the mean open times of $Ca^{2+}$ channels, while $Ca^{2+}$-channel blockers of the dihydropyridine type increase their intervals of closure [20, 21].

The contractions evoked by BAY-K-8644 in both types of vessels, and those produced by 15 mM $K^+$ in femoral ones, were abolished in $Ca^{2+}$-free medium. The vessels began to contract on $Ca^{2+}$ addition. This indicates the absolute dependence of these contractions on extracellular $Ca^{2+}$ as well as the ability of BAY-K-8644 to potentiate contractile responses caused by $Ca^{2+}$ addition in partly depolarized (femoral) or non-depolarized (cerebral) arteries. These results confirm the mechanism reported for BAY-K-8644 as a $Ca^{2+}$-activator-promoting $Ca^{2+}$ influx [4, 6, 7], being unable to show its effects in $Ca^{2+}$-free medium. The blocking effect of nifedipine on the contractile response caused by $Ca^{2+}$ in $Ca^{2+}$-free medium with BAY-K-8644, supports this interpretation of this compound's mechanism of action. When high $Ca^{2+}$ concentrations were added the arteries began to relax. This effect could be due to their suggested membrane-stabilizing effects [22, 23]. The relaxation was reduced by nifedipine, as found by Godfraind [22] in rat arteries exposed to high $K^+$ concentrations. He suggests that it is due to a $Ca^{2+}$ interaction with the cellular mechanism placed at the active site of nifedipine. It is probable that this interaction also occurs in femoral and cerebral arteries.

Preincubations with BAY-K-8644 potentiated the contractions produced by different $K^+$ concentrations in femoral arteries. This potentiation was blocked by nifedipine in a dose-dependent manner, confirming the antagonism between this drug and nifedipine. The actions of $10^{-7}$ M BAY-K-8644 and $3 \times 10^{-7}$ M nifedipine are dependent on the con-

centrations of $K^+$ used, since the higher the ion concentration the lower the effect obtained. This might be due to the fact that the number of $Ca^{2+}$-channel activated augment in proportion to the concentration of $K^+$. The contrary occurs with the ability of BAY-K-8644 to activate them further. Similar findings have been obtained in cat adrenal glands [6] in which the drug-increased catecholamine secretion was greater at moderate depolarizing stimuli. An analogous hypothesis can explain the effect of $3 \times 10^{-7}$ M nifedipine. An increase in $K^+$ concentrations will activate more of these channels, thus overcoming the inhibitory action of the $Ca^{2+}$ blocker. On the other hand, when BAY-K-8644 and nifedipine were present in the medium at the same concentration ($10^{-7}$ M), no alteration in $K^+$ elicited contractions was found, indicating that competition of both drugs antagonize mutually for the same site in equal concentrations.

The contractions evoked by NA at a concentration that produces a maximal response was not potentiated by BAY-K-8644, although the drug increased the responses induced by $K^+$ concentrations (75 and 125 mM) that also produced maximal effects. These results indicate that the $Ca^{2+}$ channels used by the amine to induce $Ca^{2+}$ entry are different to those employed by $K^+$. Indeed, it has been reported that $K^+$ induces $Ca^{2+}$ influx through voltage-sensitive $Ca^{2+}$ channels, whereas NA evokes $Ca^{2+}$ entry by means of receptor-operated $Ca^{2+}$ channels [2, 12]. All these results agree with the assumption that BAY-K-8644 and nifedipine seems to act specifically on the first type of channels but not on the second ones.

It is interesting to note that cerebral vessels are very sensitive to $Ca^{2+}$ antagonists [8–10, 24]. This work showed that they are also very sensitive to the stimulatory effects of BAY-K-8644. The possibility of manipulating the degree of activation of slow $Ca^{2+}$ channels with this drug opens new perspectives in their characterization at the level of the cerebrovascular bed.

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