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Methylflavonolamine hydrochloride inhibits contractions induced by noradrenaline, calcium and potassium in rabbit isolated aortic strips

Ming-sheng Zhang & Er-feng Zhou

Department of Pharmacology, Shanxi Medical College, Taiyuan 030001, China

1 The effects of methylflavonolamine hydrochloride (4'-methyl-7-(2-hydroxy-3-isopropylamino-propoxy)-flavone hydrochloride, MFA) were investigated and compared with verapamil and papaverine on rabbit isolated aortic strips, which were contracted by noradrenaline, calcium and potassium.

2 Pre-incubation for 25 min with either MFA (0.03 to 0.2 mM) or papaverine (0.03 to 0.2 mM) induced non-parallel and concentration-dependent rightward displacements of the curves to noradrenaline (0.00001 to 0.1 mM) with the maximal response depressed. The calculated pD'_2 values (mean ± s.d.) were 3.89 ± 0.15 for MFA and 3.93 ± 0.05 for papaverine, respectively. Verapamil (0.03 to 0.2 mM) inhibited the contraction induced by noradrenaline in a competitive manner with a pA_2 value of 5.91 ± 0.83.

3 In depolarized aortic strips of the rabbit, prior exposure to MFA (0.03 to 0.3 mM) and papaverine (0.03 to 0.2 mM) shifted the cumulative curves to Ca^{2+} (0.003 to 100 mM) parallel to the right with the maximal responses depressed, pD'_2 values being 3.88 ± 0.05 and 3.89 ± 0.13, respectively. Verapamil produced comparable inhibition of the contraction at much lower concentrations (30 to 300 nM).

4 MFA (0.03 and 0.1 mM) inhibited the contraction elicited by graded depolarization at a constant Ca^{2+} concentration with a pD'_2 value of 4.09 ± 0.07.

5 The present results show that MFA has some actions consistent with a calcium antagonist. It resembles papaverine more closely than verapamil.

Introduction

Methylflavonolamine (MFA) increases coronary blood flow, prevents experimental myocardial infarction in rabbits (Han *et al.*, 1986) and has anti-arrhythmic effects on cholorform-, $BaCl_2$- and aconitine-induced arrhythmias in rats (Han *et al.*, 1987). MFA also inhibits thrombus formation in rats and rabbits (Wu *et al.*, 1986). Studies *in vitro* have shown that MFA inhibits rabbit platelet aggregation induced by ADP, thrombin and collagen (Wu *et al.*, 1988). MFA also decreases the rate of spontaneously beating rabbit atria and depresses isoprenaline-induced positive chronotropic action in rabbit isolated atria (Zhang & Zhou, 1987). The mechanism of action of MFA is unknown, but some actions are suggestive of calcium antagonism. In an attempt to explore this mechanism, the effects of MFA were studied and compared with those of verapamil and papaverine on noradrenaline- and Ca^{2+}-induced contractions in isolated aortic strips of the rabbit.

Methods

Rabbits of either sex, weighing 1.5–2.5 kg were stunned and the thoracic aorta was rapidly excised. The aorta was cleaned of connective tissue and cut spirally into strips of 3 mm in width and 20 mm in length in cold physiological salt solution (PSS) bubbled with 100%. O_2. The two ends of the strip were ligated, connected to the transducer and the glass aerator hook, respectively, and suspended in 40 ml PSS bubbled with 100% O_2, 34°C. All strips were usually left 2 h before beginning the experiments. PSS was changed every 15–20 min during equilibration in order to prevent the accumulation of metabolites.

After equilibration, concentration-response curves to agonists were obtained by exposure of the strip to logarithmically increasing concentrations of agonist, the concentration being increased by cumulative addition of agonist to the bath after the maximal response to the previous concentration was reached.

When the two successive curves were consistent, the strip was allowed to recover for 20 min before an antagonist was added to the bath. After 25 min incubation with the antagonist, the curve was reconstructed. The strips from the same rabbit were used for different antagonists simultaneously and, moreover, one strip served to monitor the sensitivity of the strips to the agonist. If the maximal control response changed by more than 10% during the experiments, the results were discarded. Eventually, the maximal contraction was taken as 100% and pD_2, pA_2 or pD'_2 values were calculated (Arunlakshana & Schild, 1959). All experimental procedures were the same with different agonists unless stated elsewhere.

Solutions

PSS had the following composition (mM): NaCl 154.7, KCl 5.4, $CaCl_2$ 2.5, Tris 6.0 and glucose 11.0. Ca-deprived PSS was the same as PSS except 0.5 mM ethylene glycol-*bis* (beta-aminoethylester) N, N, N′, N′-tetraacetic acid (EGTA) was added instead of 2.5 mM $CaCl_2$. K-depolarizing PSS had the following composition (mM): NaCl 100.0, KCl 60.0, Tris 6.0 and glucose 11.0. Na-omitted high K-PSS was (mM): NaCl 0.0, KCl 160.0, $CaCl_2$ 2.5, Tris 6.0, glucose 11.0. All solutions were adjusted to pH = 7.40 at 34°C with 10 N HCl. In the experiment involving the cumulative addition of K, all solutions contained 10^{-6} M phentolamine in order to eliminate any effect of noradrenaline released from nerves by depolarization.

Drugs

MFA powder was provided by Shanghai Pharmaceutical Industrial Institute. The powder was either disolved in distilled water or directly disolved in PSS just before use. Verapamil was from Knoll AG and phentolamine from Ciba-Geigy. Noradrenaline and papaverine were obtained from Shenyang Pharmaceutical Co and EGTA and Tris were from Beijing Chemical Manufacturer.

Results

Effects of pre-incubation with the antagonists on noradrenaline-induced contractions

Figure 1 shows the mean cumulative concentration-response curves to noradrenaline alone and in the presence of MFA, verapamil and papaverine. At 3×10^{-5} M, noradrenaline produced the maximal contraction with a pD_2 value of 6.68 ± 0.26. At 0.03 to 0.2 mM, MFA and papaverine shifted the curves in a non-parallel fashion to the right with the maximal

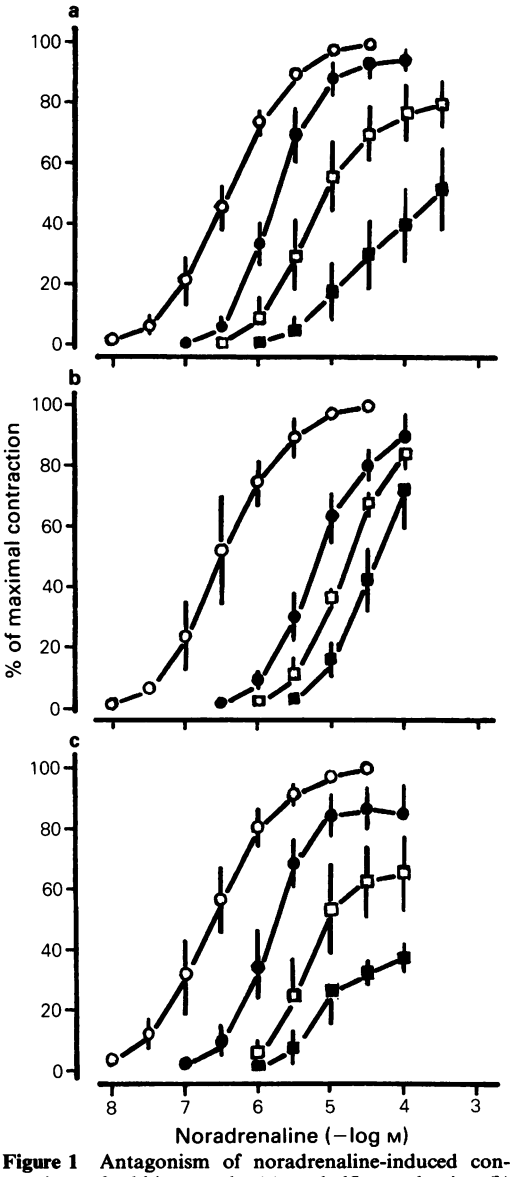

Figure 1 Antagonism of noradrenaline-induced contractions of rabbit aorta by (**a**) methylflavonolamine, (**b**) verapamil and (**c**) papaverine. The concentrations of antagonist used are 0.03 mM (●), 0.1 mM (□) and 0.2 mM (■); (○) control responses to noradrenaline. Results (mean with s.d. shown by vertical lines) are expressed as percentages of the maximal control responses obtained before addition of antagonist (n = 5–10).

responses depressed, suggesting non-competitive antagonism with the calculated pD'_2 values being 3.89 ± 0.15 and 3.93 ± 0.05, respectively. Verapamil shifted the curve to the right and in parallel. A Schild

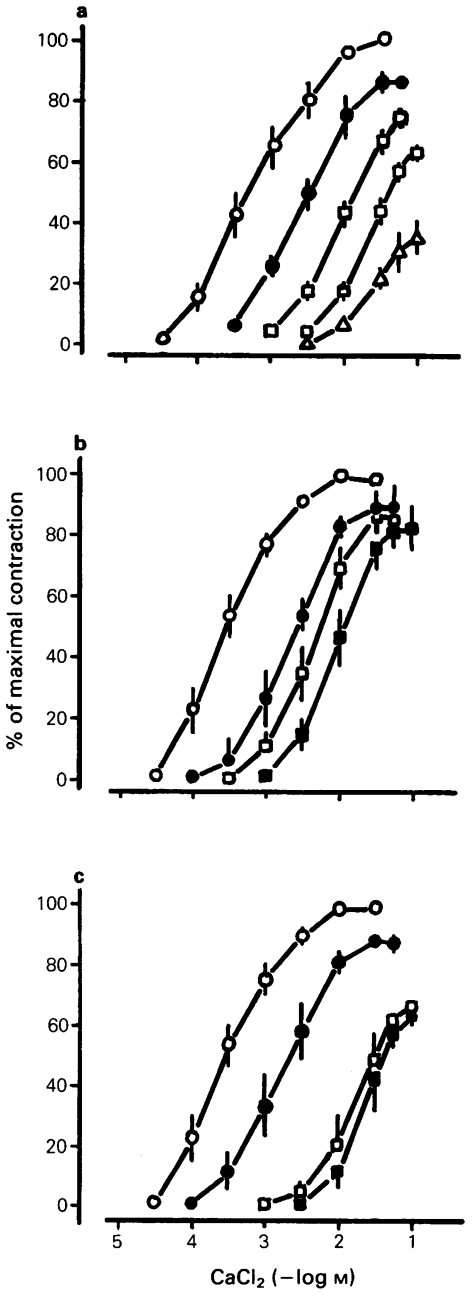

Figure 2 Antagonism of $CaCl_2$-induced contractions of depolarized rabbit aorta by (a) methylflavonolamine (● 0.03 mM, □ 0.1 mM, ■ 0.2 mM and △ 0.3 mM), (b) verapamil (● 30 nM, □ 100 nM and ■ 200 nM) and (c) papaverine (● 0.03 mM, □ 0.1 mM and ■ 0.2 mM); (○) control responses to $CaCl_2$. Results (mean with s.d. shown by vertical lines) are expressed as percentages of the maximal control contractions obtained before addition of antagonist ($n = 5$).

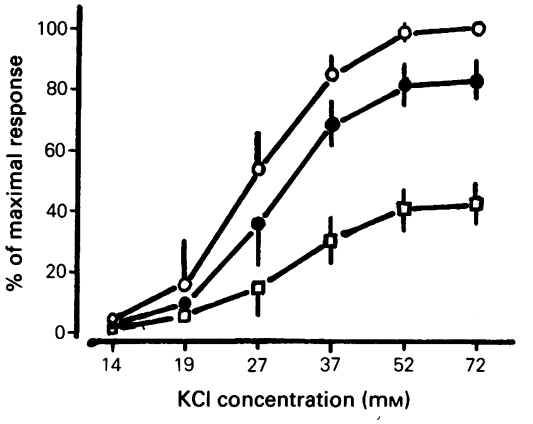

Figure 3 Inhibition of KCl-induced contraction of rabbit aorta by methylflavonolamine (● 0.03 mM and □ 0.1 mM); (○) control responses to KCl. Results (mean with s.d. shown by vertical lines) are expressed as percentages of the maximal control contraction obtained before addition of methylflavonolamine ($n = 5$).

plot of verapamil against noradrenaline yielded a straight line with a slope of -1.02 ± 0.24, suggesting competitive antagonism. The pA_2 value was 5.91 ± 0.84, which is compatible with the results reported by Motulsky *et al.* (1983).

Effect of prior exposure to the antagonists on $CaCl_2$-induced contractions in a depolarizing Ca^{2+}-free medium

After 1 h equilibration in normal PSS, the strip was rinsed 4 times with and incubated for 20 min in Ca-deprived PSS before it was rinsed 4 times with and incubated 30 min in 60 mM K^+-depolarizing PSS. $CaCl_2$ was added cumulatively to the K-depolarizing PSS, maximal tension occurring at 30 mM (Figure 2). At the concentrations mentioned above, MFA and papaverine displaced the Ca^{2+} cumulative curves in parallel and to the right with the maximal responses depressed. The calculated pD'_2 values were 3.88 ± 0.05 for MFA and 3.89 ± 0.13 for papaverine, respectively. On the other hand, verapamil produced a rightward parallel displacement of the curve at much lower concentrations (30 to 300 nM). This result meant that the inhibitory effect of verapamil on Ca^{2+}-induced contraction is nearly 1000 fold more potent than its inhibitory effect on noradrenaline-induced contraction.

Effect of MFA on contraction induced by graded depolarization

After 2 h equilibration, Na-free high K PSS was successively added to the bath containing 20 ml normal

PSS in order to elevate the KCl concentration isotonically. The maximal stimulation was reached at 72 mM (Figure 3). MFA depressed the K-induced contracture with a pD'_2 value of 4.09 ± 0.07.

Discussion

The contraction of smooth muscle is brought about by elevation of the cytosolic free Ca^{2+} level, but the sources of this activator Ca^{2+} vary with different stimuli and different types of smooth muscle (Zhang & Zhou, 1985). In the rabbit aorta, $CaCl_2$-induced contraction in a depolarizing medium depends only on extracellular Ca^{2+} influx through potential-dependent Ca channels, while noradrenaline mainly mobilizes intracellular Ca^{2+} to produce contraction (van Breemen *et al.*, 1981). MFA inhibited $CaCl_2$- and KCl-induced contractions concentration-dependently, suggesting that MFA may interfere with calcium entry through the depolarized membrane. However, comparison of the relative inhibitory potency of the drugs against noradrenaline- and Ca^{2+}-induced contractions shows that MFA is similar to papaverine, but differs significantly from verapamil, a calcium entry blocker. Verapamil selectively inhibited Ca^{2+}-induced contraction at concentrations nearly 1000 fold lower than those that antagonized noradrenaline-induced contraction, while MFA and papaverine showed no selectivity between the two agonists.

Papaverine increases the efflux of Ca^{2+} from cells and uptake of Ca^{2+} by intracellular organelles (Thorens & Haeusler, 1979; Koike & Takayanagi, 1981) and inhibits cyclic AMP phosphodiesterase (Demesy-Waeldele & Stoclet, 1975). Equal inhibition of noradrenaline-and $CaCl_2$-induced contractions in the rabbit aortic strip is also shared by other drugs which affect calcium availability intracellularly (Asano *et al.*, 1981). On the other hand, most of calcium entry blockers selectively inhibit Ca^{2+}-induced contraction (Hof *et al.*, 1984; Nyborg & Mulvany, 1984; van Breemen *et al.* 1981). Based upon our results and literature, we think it most likely that MFA inhibits contraction by reducing transmembrane Ca^{2+} influx, but we cannot exclude the possibility that MFA exerts this inhibitory effect by an action similar to that of papaverine.

Taken together, our results suggest that MFA reduces calcium availability. However, the exact sites at which MFA acts need further investigation.

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