J. Pharm. Pharmacol. 1991, 43: 356–359
Communicated November 6, 1990

Comparison of the effects of hydralazine and nifedipine on contractions and ^{45}Ca influx of rat aorta

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Abstract—The effect of the vasodilator hydralazine has been compared with nifedipine on KCl-(K^+) (60 mM) and noradrenaline-(NA) (10 μM) induced ^{45}Ca uptake and contractile responses in rat aorta arterial strips without endothelium. Hydralazine (0·5–10 mM) was equally effective in relaxing K^+- (IC50 = 2·2 ± 0·17 mM) and NA- (IC50 = 3·06 ± 0·25 mM) induced tension, the degree of relaxation depending on the dose. Nifedipine totally inhibited K^+- (IC50 = 3·16 ± 0·28 nM) induced contractions with lower doses than were necessary to relax (up to 54·0 ± 4·1% with supramaximal concentrations) NA-induced contractions (IC50 = 1·48 ± 0·12 μM). In the experiments in a calcium-free medium, nifedipine (1 μM) had no effect on the NA- (10 μM) induced contractions whereas hydralazine (1 mM) strongly inhibited them. Nifedipine did not affect the basal uptake of ^{45}Ca but the induced uptakes were reduced to 66·3 ± 3·2% (K^+) and 65·5 ± 4·1% (NA) of their basal values. Hydralazine did not affect the basal uptake of ^{45}Ca nor that induced by the two vascoconstrictor agents. These results suggest that nifedipine acts on the cell membrane by blocking the movements of calcium through the voltage-dependent and receptor-operated calcium channels, whilst hydralazine has an intracellular effect.

It is commonly accepted that hydralazine directly relaxes the vascular smooth muscle (Khayyal et al 1981; Lipe & Moulds 1981; Brown et al 1983; Alonso et al 1987; Orallo et al 1991) through an unknown mechanism (Cano et al 1986; Eleno et al 1987). In 1974, Limas & Cohn proposed that hydralazine could act by stimulation of an electrogenic Na-K-pump, thereby leading to hyperpolarization and a subsequent vasodilatation. More recently, a hydralazine-induced hyperpolarization of 4 mV was described for the rat tail artery (Hermsmeyer et al 1983). However, in contrast to several vasodilators like diazoxide, minoxidil sulphate, cromakalim, pinacidil and nicorandil (for review see Cook & Quast 1990), the ability of hydralazine to open Ca^{2+}-dependent K^+ channels in vascular smooth muscle has not yet been shown.

It is known that second messengers such as cyclic AMP (cAMP) and cyclic GMP (cGMP) activate specific cAMP-dependent protein kinase and cGMP-dependent protein kinase, respectively. These protein kinases phosphorylate myosin light chain kinase from vascular and non-vascular smooth muscle to induce relaxation (Kamm & Stull 1989). Also, the cyclic nucleotides cAMP and cGMP may alter smooth muscle contraction by decreasing intracellular Ca^{2+} concentrations via phosphorylation of calcium channels or stimulation of Ca-ATPases in the plasmalemma and sarcoplasmic reticulum (van Breemen & Saida 1989). In 1973, Andersson observed that hydralazine stimulates adenylate cyclase and increases cAMP content in bovine mesenteric artery. However, Diamond & Janis (1978) reported that increases in cGMP levels are not responsible for the relaxant effects of hydralazine in rat vas deferens.

Studies by McLean et al (1978) and Weiss et al (1981) showed that hydralazine may interfere with transmembrane ^{45}Ca influx. However, data which argue against a cell membrane mechanism of action for hydralazine have also been reported (Diamond & Janis 1980; Diamond & Shaikh 1980). Those investigators suggest that at least high concentrations of hydralazine may act at a step in the excitation-contraction coupling sequence beyond the regulation of cytoplasmic Ca^{2+} levels, while Lipe & Moulds (1981) demonstrated that hydralazine interferes with the release of more tightly bound calcium in human arteries, possibly from intracellular storage sites.

In view of those reports, in the present work the effects of hydralazine on tension responses to K^+ and noradrenaline (NA) and on ^{45}Ca uptake in rat aorta have been investigated and compared with the corresponding effects of the calcium antagonist nifedipine.

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Materials and methods

Rat isolated thoracic aorta. Male Sprague-Dawley rats, 250–350 g, were killed by a blow on the head. The thoracic aorta was rapidly removed, deprived of endothelium by running a glass stick through the lumen, cut into small spiral strips (3–4 cm long and 1 mm wide) and immediately transferred to an organ bath with a Krebs solution containing (mM): NaCl 118, KCl 4·7, $MgSO_4.7H_2O$ 1·2, $CaCl_2.2H_2O$ 2·5, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 11, thermoregulated at 37 °C and bubbled with 95%-O_2 5% CO_2. Calcium-free solution was prepared by omission of calcium when required. After an equilibration period of at least 1 h under 1 g resting tension, isometric contractions induced by NA (10 μM) or K^+ (60 mM), without keeping the osmolarity constant, were recorded through a force-displacement transducer (Letica) connected to a polygraph (Letica 1000-100) for 15 min. Cumulative doses of hydralazine or nifedipine were then added, and the effect of each one observed for 10 min.

To obtain contractions in a calcium free medium, artery preparations were equilibrated for 60 min in normal Krebs solution and then washed three times over a 20 min period with a calcium-free solution (containing 0·2 mM EGTA) before a vasoconstrictor agent contraction was elicited. To study the effects of hydralazine and nifedipine, the preparations were further washed in normal Krebs solution for 60 min (to fill the Ca^{2+} stores depleted by the first contraction). There was a further 20 min pre-incubation in calcium-free solution before a suitable concentration of hydralazine or nifedipine was added, followed 10 min later by NA. Other tissues were subjected to the same procedure simultaneously, but omitting nifedipine and hydralazine.

^{45}Ca influx. Aortic rings weighing 5–9 mg were equilibrated for at least 60 min in physiological solution (composition mM: NaCl 139, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1·5, Hepes 5, glucose 10), maintained at 37 °C and aerated with 100% O_2. Afterwards, the tissues were incubated for 5 min in a ^{45}Ca- (New England Nuclear, specific activity 35 mCi mg^{-1}) containing medium (0·6 μCi mL^{-1}) with or without NA (10 μM) or K^+ (60 mM) to analyse the effect of these vasoconstrictor agents on ^{45}Ca uptake. To investigate the actions of hydralazine and nifedipine on this uptake, they were added to the bath 20 min before and during the incubation period with ^{45}Ca. Thereafter, the preparations were washed for 45 min in 500 mL of an ice-cold Ca-free physiological solution containing 2 mM EGTA, pH 7·4, bubbled with 100% O_2 to remove extracellular Ca^{2+} from the tissue. Afterwards the arteries were blotted, weighed and digested in 1 mL H_2O_2 (110

volumes) at 115°C for 90 min. After cooling, 5 mL of Ready-Safe Beckman was added and the radioactivity of the samples counted in a liquid scintillation counter (Beckman LS 3801).

The ^{45}Ca tissue uptake was calculated as follows: ^{45}Ca uptake (nmol ^{45}Ca/kg wet tissue) = counts per min in tissue/wet tissue weight (kg) × nmol ^{45}Ca in 1 L solution/counts per min in 1 L solution. Unlike other authors, to avoid inaccuracy in the calculation of ^{45}Ca uptake, 1·5 mM Ca^{2+} present in physiological solution was not included in the nmol ^{45}Ca in 1 L solution factor of this expression.

Statistical analysis. Unless specified, results shown in the text and figures are expressed as means ± s.e.m. Statistical differences between two means ($P < 0.05$) were determined by Student's two-tailed *t*-test for paired and unpaired data.

From the cumulative dose-response curves for the relaxant effects of hydralazine and nifedipine, the 50% inhibitory concentrations (IC50) were calculated.

Drugs and chemicals. The drugs used were: (−)-noradrenaline bitartrate (Sigma), hydralazine hydrochloride (Ciba Geigy) and nifedipine (Bayer). Hydralazine was prepared in de-ionized water immediately before use. Nifedipine was dissolved in 95% ethanol to make a stock solution of 10 mM and portions of this solution were then diluted to 10 nM with deionized water. NA was prepared daily with de-ionized water from a stock solution (100 mM) kept at 20°C. Sodium bisulphite (0·2%) was added to prevent oxidation. All the experiments with nifedipine were carried out in the dark because of the photosensitivity of this drug. The chemicals used for the preparation of the physiological solutions were of analytical grade.

Results

Vascular reactivity in normal Krebs solution. Noradrenaline (NA) (10 μM) and high potassium (high K^+) (60 mM) produced a sustained contracture in the isolated aorta arterial strips, reaching 447 ± 6·2 and 657 ± 8·1 mg, respectively (n = 10). These contractures were considered the maximum response (100%).

Hydralazine (0·5–10 mM) completely inhibited, in a dose-dependent and non-specific manner the contractions induced by NA (IC50 = 3·06 ± 0·25 mM) and by K^+ (IC50 = 2·2 ± 0·17 mM) (Fig. 1). No significant differences between IC50 values were obtained (n = 5, $P > 0.05$).

Nifedipine (1–10 nM) totally inhibited K^+-induced contractions (IC50 = 3·16 ± 0·28 nM) at lower concentrations than were necessary to inhibit (up to 54·0 ± 4·1% with supramaximal concentrations) those induced by NA (IC50 = 1·48 ± 0·12 μM, n = 5, $P < 0.01$) (Fig. 2).

Vascular reactivity in calcium-free Krebs solution. NA (10 μM) produced a characteristic contraction with two distinct components: an initial transient contraction (fast component) with tension = 132 ± 8 mg (n = 10) that relaxed to a sustained tension of 40·7 ± 3 mg (slow component). These contractions were practically unaffected by the addition of 1 μM nifedipine (fast component tension = 126 ± 8 mg, slow component tension = 38·4 ± 3·5 mg, n = 5, $P > 0.05$) whereas both components were strongly inhibited by 1 mM hydralazine (fast component tension = 40·9 ± 5 mg, slow component tension = 14·2 ± 2·5 mg, n = 5, $P < 0.01$).

^{45}Ca uptake. The calcium uptake for the segments of rat aorta in the absence of other agents (basal uptake) was 8·89 ± 0·2 nmol kg^{-1} (n = 10). The addition of nifedipine (10 nM) and hydralazine (1 mM) did not affect this value significantly (^{45}Ca tissue content: 8·63 ± 0·48 and 8·80 ± 0·37 nmol kg^{-1}, respectively, n = 5, $P > 0.05$).

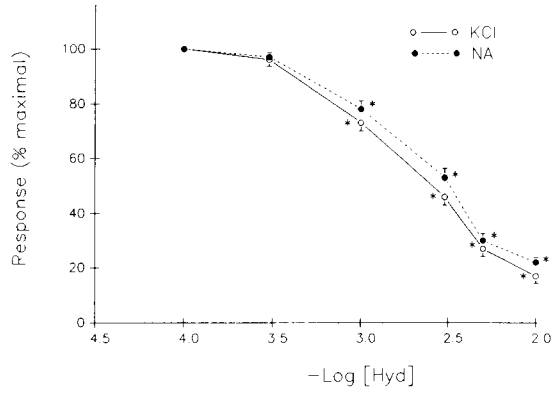

FIG. 1. Effects of hydralazine (Hyd) on noradrenaline- (NA) (10 μM)- and KCl- (60 mM)-induced contractions in rat rubbed aortic strips. Data given as a mean ± s.e.m. (n = 5). *$P < 0.05$, with respect to the maximum tension.

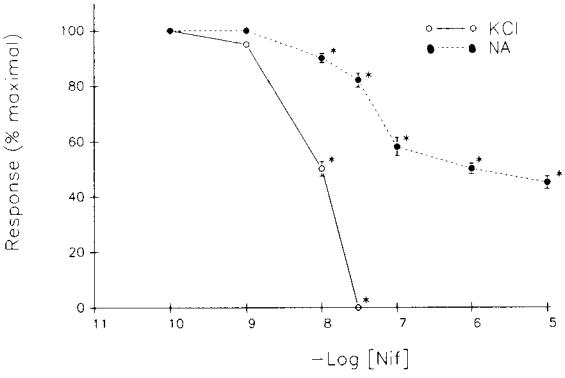

FIG. 2. Endothelium-free rat aorta: effects of nifedipine (Nif) on noradrenaline- (NA) (10 μM) and KCl- (60 mM) induced contractions. Data are plotted as a mean ± s.e.m. (n = 5). *Level of statistical significance $P < 0.05$, with respect to the maximum tension.

The vasoconstrictor agents NA and high K^+ considerably increased the basal ^{45}Ca uptake (^{45}Ca tissue content: 12·47 ± 0·8 nmol kg^{-1} (NA) and 15·07 ± 0·71 nmol kg^{-1} (K^+), n = 10, $P < 0.01$).

Nifedipine (10 nM) produced an inhibition of NA- and high K^+-induced ^{45}Ca uptake (tissue content of ^{45}Ca: 10·12 ± 0·29 and 10·97 ± 0·33 nmol kg^{-1}, n = 5, $P < 0.05$), whereas hydralazine (1 mM) had no significant inhibiting action (tissue content: 11·05 ± 0·86 nmol kg^{-1} (NA) and 13·51 ± 0·68 nmol kg^{-1} (high K^+), n = 5, $P > 0.05$) (Fig. 3).

Discussion

It has been reported that high K^+ causes marked contractions in rat aortic tissue by depolarizing the smooth muscular cells and increasing the influx of calcium through L-voltage-dependent channels (Bean et al 1986; Tsien et al 1988; Godfraind & Govoni 1989). Activation of α_1-adrenergic receptors by NA in rat aorta induces a two-phase contraction: an initial transient contraction, caused by the release of calcium from intracellular stores (via inositol trisphosphate) and a slow and sustained contraction, due to Ca^{2+} influx through the receptor-operated Ca^{2+} channel (Putney 1986; Karaki & Weiss 1988). The results presented here show that nifedipine inhibits K^+-induced contractions at lower concentrations than are required to relax contractions induced by NA. This supports previous studies (Godfraind 1983), suggesting that nifedipine mainly acts on the

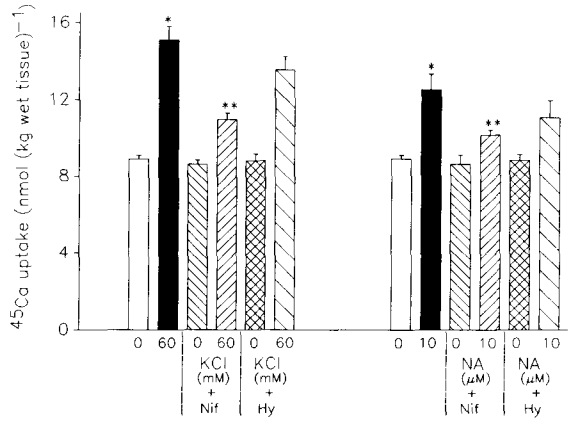

FIG. 3. Effects of nifedipine (Nif) (10 nM) and hydralazine (Hy) (1 mM) on ^{45}Ca uptake induced by noradrenaline (NA) and KCl in rat aortic rings without endothelium. Error bars on the columns show s.e.m. of five experiments. $*P < 0.01$ with respect to basal ^{45}Ca uptake. $**P < 0.05$ with respect to ^{45}Ca influx induced by NA or K^+.

L-type voltage-dependent channel. Hydralazine, however, relaxes with equal effectiveness K^+- and NA-induced contractions, inhibiting them almost completely, which suggests that its primary action occurs within the cell, as reported for human arteries (Lipe & Moulds 1981). A possible secondary, non-selective mechanism of action of hydralazine acting on the cell membrane, blocking calcium influx through voltage-dependent and receptor-operated channels is in agreement with previous studies in rat tail artery (Khayyal et al 1983). Cromakalim and other potassium channel openers relax contractions to low (< 25 mM) but not high (> 30 mM) KCl in various vascular preparations (Hamilton et al 1986; Clapham & Wilson 1987). However, hydralazine is able to relax high potassium (60 mM) contractions. This suggests that hydralazine does not exert its vasorelaxant effects by opening membrane K^+ channels in rat aorta.

Further evidence for the action of nifedipine on calcium channels is given by the experiments involving ^{45}Ca. The basal value of ^{45}Ca uptake, and consequently the amount of calcium entering by means of the leak channels (Hurwitz 1986; Karaki & Weiss 1988), is unchanged by the addition of nifedipine whereas nifedipine strongly inhibits the uptake of ^{45}Ca induced by K^+ and NA, as has been observed previously (Godfraind 1983; Godfraind & Miller 1983), suggesting that nifedipine blocks transmembrane calcium influx. Hydralazine does not reduce basal uptake of ^{45}Ca nor the uptake induced by NA and K^+, contrary to results obtained by McLean et al (1978) and by Weiss et al (1981) for rabbit aorta. This suggests that hydralazine does not block the transmembrane calcium movements through voltage-dependent and receptor-operated calcium channels in rat aorta. The differences in the present results from those of McLean et al (1978) and Weiss et al (1981) may be due to the fact that those authors used rabbit aorta. It has been reported that, in rabbit aorta, the contractions induced by several vasconstrictor agents are less dependent on extracellular calcium in this tissue than they are in rat aorta (Godfraind 1988). On the other hand, several differences in the properties of the vascular smooth muscle cell membrane have been described (Vanhoutte et al 1983). However, the selective effects of hydralazine to inhibit ^{45}Ca influx in some vascular preparations, as well as many observations relating to Ca^{2+} antagonist selectivity (Cauvin et al 1988), remain unexplained.

The effects of hydralazine within the cell are shown from the results with calcium-free medium. Addition of NA in the absence of external Ca^{2+} induces a fast and transient contraction attributed to release of intracellular Ca^{2+} stores followed by a smaller slow and sustained contraction, whose mechanism is not clear (Godfraind & Kaba 1969; Karaki & Weiss 1988). It has been shown that activation of protein kinase C by diacylglycerol derived from phosphoinositide breakdown induces a sustained contraction in the presence of a low concentration of Ca^{2+} (Nishizuka 1984). In this work, the results in a calcium free medium show two main findings:

(i) that nifedipine, in agreement with Dacquet et al (1987), does not act within the cell because it does not inhibit NA-induced contractions in a calcium-free medium.
(ii) that hydralazine acts intracellularly as described by Lipe & Moulds (1981) in human arteries and veins, because it inhibits both phases of the contraction elicited by NA in a calcium-free solution. Thus it may inhibit the release of intracellular calcium induced by inositol triphosphate, act directly on the contractile apparatus as previously reported by Jacobs (1984) in bovine carotid arteries, or activate the calcium uptake into intracellular stores. It is unlikely that this intracellular action is due to an increased rate of calcium loss (directly or indirectly via increase of cyclic nucleotides) as hydralazine does not stimulate calcium-dependent ATPase in rat aorta (Eleno et al 1987).

In conclusion, both nifedipine and hydralazine have been characterized as agents with clear vascular smooth muscle actions, but the cellular basis of the mechanisms by which the two agents act differs considerably.

This work was supported in part by grants from the Xunta de Galicia, Conselleria de Educación (Spain). We are very grateful for the generous supplies of hydralazine hydrochloride by Ciba-Geigy and nifedipine by Bayer.

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