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Research Article

Vascular effects of 3-carbomethoxypyridine on rabbit aortic smooth muscle

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3-Carbomethoxypyridine, Rabbit Aorta,Vascular smooth muscle, Endothelium

ABSTRACT

Background: 3-Carbomethoxypyridine (3-CMP) is a methyl nicotinate that has been isolated and characterized from one of the alkaloidal fractions of Pyrenacantha staudtii. No literature is available on its vascular action. The goal of this study was to characterize the mechanism of action of 3-CMP on rabbit aortic smooth muscle. **Methods:** Isometric contractions of ring segments of rabbit aorta (under an initial load of 2g) suspended in 20ml organ baths containing physiological salt solution (PSS) and bubbled with 95% O_2, 5% CO_2, were examined at 37^0C and pH 7.4. The protocols examined are: Dose response of tissues to phenylephrine (PE), effect of 3-CMP on baseline tension, dose response of tissues to 3-CMP following phenylephrine (PE, 10^{-7}M) or high K^+ (40mM) pre-contraction as well as relaxation responses to 3-CMP and Ach in endothelium-intact and endothelium-denuded rings. **Results:** The results show that 3-CMP dose-dependently attenuated the contractile responses to PE and High K^+. The respective maximum relaxation responses to 3-CMP following pre-contractions with PE (n=8) or high K^+ (n=9) were 50.06±2.94 and 18.59±2.88 ($p<0.05$). Ach-induced relaxation was observed only in rings with intact endothelium. Also 3-CMP-induced relaxation responses were significantly attenuated in endothelium-denuded rings. **Conclusion:** The results suggest that 3-CMP elicits relaxation of rabbit aortic smooth muscle activated by depolarization-dependent (high-K^+) or –independent (PE) agents. The greater effect on PE contraction suggests interference with mechanisms involving agonist-receptor interaction. 3-CMP relaxation is also endothelium-dependent.

INTRODUCTION

3 – carbomethoxypyridine (3 – CMP) is an alkaloidal enterocyclic compound (Fig. 1). It is a methyl nicotinate, which is pale yellow to brown crystals (Falodun and Usifoh, 2006). 3-CMP was isolated and characterized from one of the active fractions of the methanolic extract of leaves of Pyrenacantha Staudtii - an annual herb found in the tropical forest and farmland bushes (Falodun and Usifoh, 2006). Pyrenacantha Staudtii plant is used in folk medicine for the treatment of stomach colic, dysmenorrhea and threatened abortion (Agbakwuru et al, 1988; Mesia et al, 2005). There are reports showing that the leaves have anti-malarial activity (Mesia et al, 2005). Also, aqueous extracts of the leaves have been shown to reduce gastric ulcer in experimental animals Aguwa and Mittal, 1978). A recent study by Falodun et al (2009) reported that the crude methanol extract of Pyrenacantha staudtii has a relaxant effect on isolated rat ileum. 3-CMP also inhibits rat uterine smooth muscle contraction Nworgu et al, 2007). There is a paucity of information in the literature on the cardiovascular effect of 3-CMP. The goal of this study was to characterize the mode of vascular action of 3-CMP on isolated ring preparations of rabbit aorta.

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METHODS

3-Carbomethoxypyridine:

3-CMP was synthesized and characterized by the Department of Pharmaceutical Chemistry, of the

University of Benin. Fresh dilution was made with distilled water for each experiment.

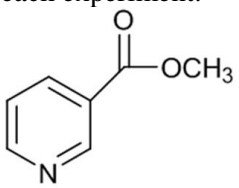

Fig. 1
Structure of 3-Carbomethoxypyridine (3-CMP)

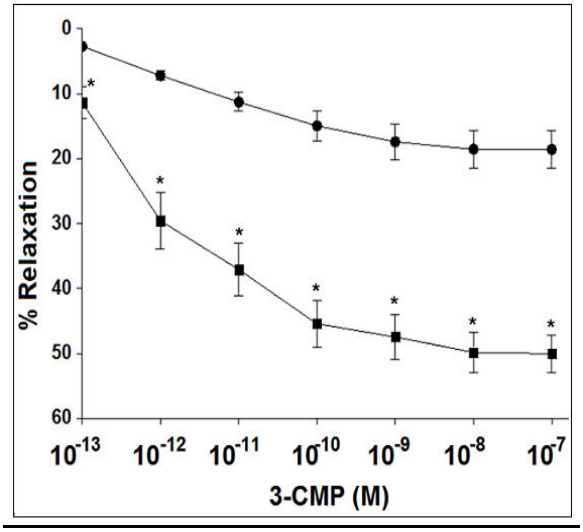

Fig. 2
Comparison of the relaxation responses to cumulative addition of 3-CMP following pre-contraction induced by PE (■) or high K^+ (●). Values are means ± SEM: n=8. P<0.05

Tissue Preparation:
Segments of the aorta were obtained from New Zealand rabbits, sacrificed by stunning. The tissues were placed in Physiological salt solution (PSS), carefully cleaned free of all adhering connective tissues and cut into 2mm rings. The tissues were suspended between 2 L-shaped wire loops in 20ml organ baths containing PSS. The upper loop was attached to a Grass Model FT03 force transducer connected to a Grass Model 7P polygraph (Grass Instruments Co., Quincy, MA, USA) while the lower loop was fixed to the base of the organ bath. The composition of the PSS was (mM/L): NaCl 119, KCl 4.7, $NaHCO_3$ 24.9, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.6, glucose 11.5. The PSS was bubbled throughout with 95% O_2 - 5% CO_2 gas mixture with the pH and temperature maintained at 7.4 and 37°C respectively. High-K^+ PSS was prepared by equimolar replacement of K^+ with Na^+. Aortic rings were given a resting tension of 1g. We have previously reported (Ebeigbe and Cabanie, 1991; 1992) that this level of initial tension produces maximal active contractions in rings stimulated with noradrenaline or a depolarizing solution. An equilibration period of 90 minutes was allowed.

Experimental Protocols
Response to high-K^+ PSS
Followin g equilibration, aortic rings were made to contract twice with 80mM K^+, at 20-minute interval. The average of these contractions represented the maximum (100%) against which subsequent contractions were evaluated Ebeigbe and Cabanie, 1992; Ojeikere et al, 2003; Omogbai et al, 2005).

Dose-Response to Phenylephrine:
Phenylephrine was added to the bath cumulatively; a higher concentration was added when the response to the previous concentration has stabilized. The contractions were matched against the reference (100%) contraction induced by high-K^+ (80mM) depolarizing solution.

Effect of 3-CMP on baseline tension
3-CMP was added to the bath cumulatively, to examine the possible contractile effect of the compound.

Dose response to 3-CMP
Aortic rings were contracted with EC_{50} concentrations of PE ($1x10^{-7}$M) or high K^+ (40mM). At the peak of the contraction, 3-CMP was added to the bath cumulatively. The relaxation responses were compared against the PE or high-K^+ pre-contraction.

Role of the endothelium;
The relaxation responses to Ach and 3-CMP in PE precontracted rings were assessed in endothelium-intact and endothelium-denuded rings. Endothelium removal was effected by gently rubbing the internal surface of the rings with a roughened glass rod (Ebeigbe and Cabanie, 1992). The effectiveness of the denudation process was confirmed by the failure of 10^{-5}M Ach to elicit relaxation in endothelium-denuded (Ebeigbe and Cabanie, 1992; Furchgott and Zawadzki, 1980).

Analysis of data
Data are presented as means ± SEM. Statistical analysis was by means of MicroCal Origin software and Student's *t*-test. A *p* value less than 0.05 was considered significant, while *n* values denote number of animals from which vessels were obtained. Tests were carried out on at least six vessel preparations. EC_{50} (concentration producing 50% of maximal contraction) values were derived graphically.

RESULTS
Response to high-K^+ PSS
Contractile responses induced by 40 and 80mM K^+ were 1134±28.60 and 1865.50 ± 49.4 mg (n=12) respectively.

Dose-Response to Phenylephrine:
Phenylephrine elicited concentration-dependent contractile responses in all experiments (n=12). The maximum contraction induced by PE was 1832±37.6mg while the EC_{50} (M) of PE contraction is 1.02 x 10^{-7}M.

Effect of 3-CMP on baseline tension
In resting (unstimulated) aortic rings, cumulative increases in 3-CMP concentration had no effect on baseline tension (n=12).

Relaxation response to 3-CMP
Following pre-contraction induced by 1.02 x 10^{-7}M PE or 40mM K^+, addition of 3-CMP cumulatively (n=8), resulted in concentration-dependent relaxation responses, The responses were significantly attenuated in K^+-contracted rings (Fig. 2).

A comparison of the magnitudes of relaxation (Fig. 3) of PE pre-contracted rings shows that although the pattern of relaxation responses to Ach and 3-CMP was similar, Ach relaxation was significantly ($p<0.05$) greater than that for 3-CMP: 44.6±2.2 and 35.4±1.5 %, respectively.

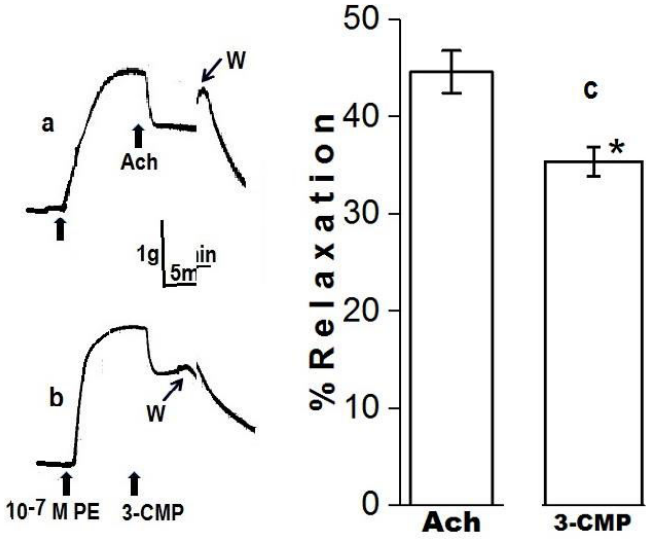

Fig. 3
Representative tracings showing relaxation response to Ach (a) and 3-CMP (b) in aortic rings pre-contracted with 10^{-7}M phenylephrine. Data are summarized in (c) for 3-CMP (n=6) and Ach (n=6). 'W' indicates tissue rinse while asterisk denotes significant difference ($p<0.05$)

Role of the endothelium:
In all experiments (n=12) Ach did not elicit relaxation in endothelium-denuded rings. In endothelium-intact rings, both Ach and 3-CMP produced concentration-dependent relaxation responses. Fig. 4 shows the relaxation responses induced by single application of Ach (1x10^{-7}M, n=6) or 3-CMP (1x10^{-6}M, n=6) in PE pre-contracted endothelium-intact rings. Ach relaxation was significantly greater ($p<0.05$).

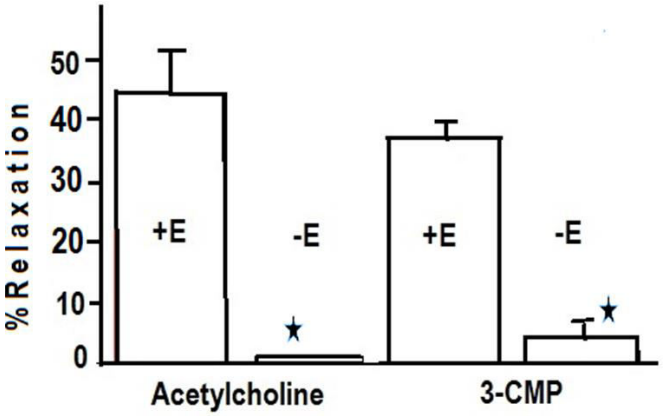

Fig. 4
Relaxation responses to 10^{-7}M Ach and 10^{-6}M 3-CMP following pre-contraction by 10^{-7} M phenylephrine in endothelium-intact (**+E**) and endothelium-denuded (**-E**) aortic rings (n = 6). ★Denotes significant difference from respective **+E** values

In endothelium-denuded rings, Ach produced no relaxation whereas 3-CMP relaxation was significantly attenuated (Fig. 4)

DISCUSSION

The results show that 3-CMP has no contractile effect on rabbit aortic smooth muscle since it did not alter baseline tension. Following pre-contraction induced by PE or high K^+ however, 3-CMP elicited dose-dependent relaxation responses; this suggests that active tone is required to reveal the vascular action of 3-CMP as have been reported for various other vasorelaxant agents (Ebeigbe and Cabanie, 1991; 1992; Ojeikere et al, 2003; Omogbai et al, 2005; Ebeigbe and Aloamaka, 1985; 1987). A relaxant effect of 3-CMP was observed on pre-contractions induced by phenylephrine, an α-adrenoceptor agonist and high K^+ PSS. These two agents are known to induce vascular smooth muscle contraction via different mechanisms: whereas phenylephrine activates α-adrenoceptor and stimulates Ca^{2+} entry via receptor operated channels as well as mobilization from intracellular stores, high-K^+ contraction involves membrane depolarization and Ca^{2+} influx through voltage-gated channels (Ebeigbe and Cabanie, 1991; 1992; Bolton, 1979). The observation that 3-CMP had greater relaxant effect on PE-contracted rings suggests that 3-CMP action is mediated through interference with mechanisms associated with agonist-receptor interaction.

Considering the importance of the endothelium in modulation of vascular smooth muscle responses (Ebeigbe and Cabanie, 1992; Furchgott and Zawadzki, 1980), we have compared the relaxant effect of 3-CMP with that of Ach – a prototype endothelium-dependent

vasorelaxant Furchgott and Zawadzki, 1980; Grygleski et al, 1986). Both Ach and 3-CMP elicited relaxation responses only in aortic rings with intact endothelium. While Ach failed to induce relaxation in endothelium-denuded rings, 3-CMP-induced relaxation was significantly attenuated. These observations suggest that the relaxant effect of 3-CMP is due, at least in part, to the release of some endothelium-derived relaxant.

In conclusion, this study shows that 3-CMP-induced relaxation of rabbit aortic smooth muscle is endothelium-dependent and also, perhaps, associated with interference with α-adrenoceptor activation.

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