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VASCULAR EFFECTS OF KETAMINE IN ISOLATED RABBIT AORTIC SMOOTH MUSCLE

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Summary: The precise mechanism by which ketamine induces relaxation of vascular smooth muscle is not clear. The goal of this study was to further characterize the vascular actions of ketamine in rabbit aortic smooth muscles. Ring segments (2mm) of rabbit aortae were suspended in 20ml organ baths containing physiological salt solution (PSS) and isometric contractions were recorded at 37^0C and pH 7.4. The medium was bubbled with 95% O_2, 5% CO_2, and rings were given an initial load of 2g. An equilibration period of 90 minutes was allowed. Three protocols were examined: (a) Effect of ketamine on baseline tension (b) relaxation-responses to ketamine following precontractions induced by 10^{-7}M phenylephrine or high K^+ (40mM) PSS and (c) Influence of presence or absence of endothelium on the relaxation response to ketamine. Ketamine produced relaxation of contractile responses induced by both phenylephrine and High K^+. The respective maximum relaxation responses induced by ketamine following precontractions by phenylephrine and high-K^+ were 76.8 ± 2.3 and 71.2 ± 8.0 ($p > 0.05$). Ach-induced relaxation was observed only in rings with intact endothelium whereas ketamine-induced relaxation was observed in intact as well as endothelium-denuded rings; this suggests that ketamine-induced relaxation of rabbit aortic smooth muscle is independent of vascular endothelium.
Key Words: *Ketamine; Vascular Smooth Muscle; Rabbit Aorta*

Introduction

Ketamine is a non-barbiturate anaesthetic agent which has been reported to have a wide variety of cardiovascular effects: alteration of systemic arterial pressure with significant increases in heart rate, cardiac output, cardiac work and myocardial oxygen requirement in normal humans (Kreusche et al, 1967) as well as hypertensive properties (Hug, 1979). Also, ketamine has been demonstrated to produce biphasic blood-pressure responses (hypotension and hypertension) in humans, rats and dogs (Domino et al, 1965, Virtue et al 1967; Dowdy and Kaya, 1968), or profound hypotension in rabbits (Clanachan et al, 1976). The mechanisms of the hypertensive actions are thought to be via the central nervous system, baroreceptors and/or vascular sympathetic neurotransmission (Fukuda et al, 1986) whereas the hypotension is thought to be caused by a direct effect on vascular smooth muscles. The inhibitory effects of ketamine on vascular smooth muscle contraction have been shown to be due to an interference with transmembrane Ca^{2+} influx (Altura et al 1980; Fukuda et al, 1983) while other mechanisms, such as effects on contractile proteins and on intracellular calcium stores, were not excluded. The goal of the present study was to examine further, the *in vitro* vascular effects of ketamine in rabbit aortic smooth muscle.

Materials and Methods

Tissue Preparation:

New Zealand rabbits were sacrificed by stunning. Segments of the abdominal aorta were obtained, cleaned free of adhering connective tissues and cut into 2-3mm rings. The rings were placed between L-shaped wire loops in 20ml organ baths containing physiological salt solution (PSS). The lower loop was attached to the base of the organ bath while 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). 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^oC respectively. High-K^+ PSS was prepared by equimolar replacement of KCl with NaCl. The rings were given a resting tension of 2g and an equilibration period of 90 minutes was allowed.

Protocol:

The following protocols were examined: (a) Effect of ketamine on baseline tension (b) relaxation-response to ketamine following precontractions induced by EC_{70} concentrations of phenylephrine (1×10^{-7}M) or KCl (40mM) and (c) relaxation response to

ketamine in pre-contracted endothelium-intact and endothelium-denuded rings.

The effects of ketamine were studied by cumulative additions of increasing concentrations of the drug into the organ bath; a next dose was added when the effect of the previous dose has been established. Endothelium removal was effected by gently rubbing the intimal surface with a pair of forceps. The effectiveness of the endothelium-removal procedure was confirmed by a lack of acetylcholine-induced relaxation in phenylephrine pre-contracted rings (Furchgott and Zawadzki, 1980).

Statistical analysis: Data are presented as means ± SEM. Statistical analysis was by means of 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. EC_{70} (concentration producing 70% contraction) values were derived graphically. The magnitude of ketamine-induced relaxation was estimated as a percentage of the pre-contraction.

Results

Effects of Ketamine on Baseline tension
In unstimulated rabbit aortic rings, cumulative additions of ketamine did not produce any observable response in all experiments (n = 10).

Effects of Ketamine on Precontracted rings
Following pre-contractions induced by phenylephrine or high-K^+ PSS, cumulative additions of ketamine resulted in concentration-dependent relaxation responses (Figs 1 and 2). The magnitudes of ketamine-induced relaxation were not significantly different, following both modes of pre-contraction. The maximal relaxation responses in phenylephrine- or high-K^+-contracted rings were (respectively): 78.3 ± 2.3 and 71.2 ± 8.0% ($p > 0.05$).

Influence of the endothelium
Fig. 3 shows our observation on the influence of the endothelium on ketamine-induced relaxation. Whereas acetylcholine-induced relaxation was completely abolished following endothelium removal, ketamine-induced relaxation persisted in endothelium-denuded rings. Fig 3c summarizes the magnitudes of relaxation in response to acetylcholine and ketamine.

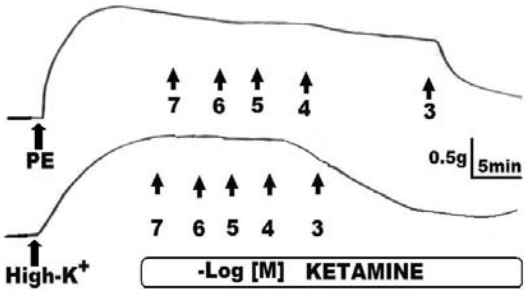

Fig. 1: Typical tracings showing the relaxation responses induced by ketamine following precontraction induced by either 10^{-7}M phenylephrine (PE) or High-K^+ (40mM) PSS

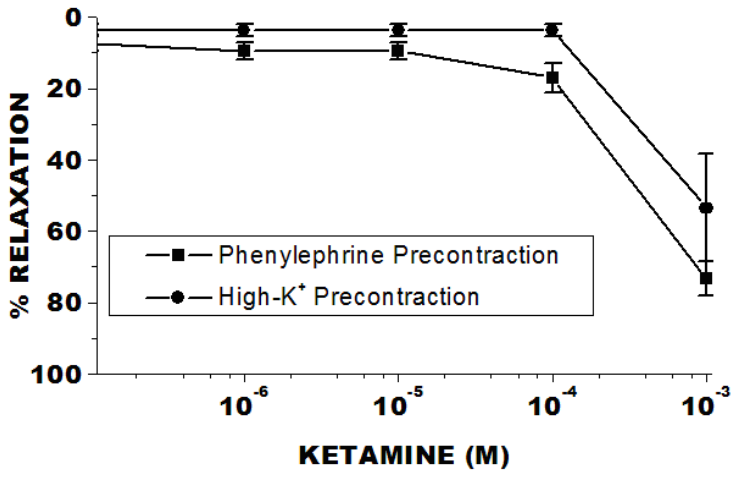

Fig. 2:Relaxation responses by ketamine following pre-contractions by 10^{-7}M phenylphrine or 40mM K^+ PSS. Values are means ±SEM; n = 9. The two curves are not significantly different.

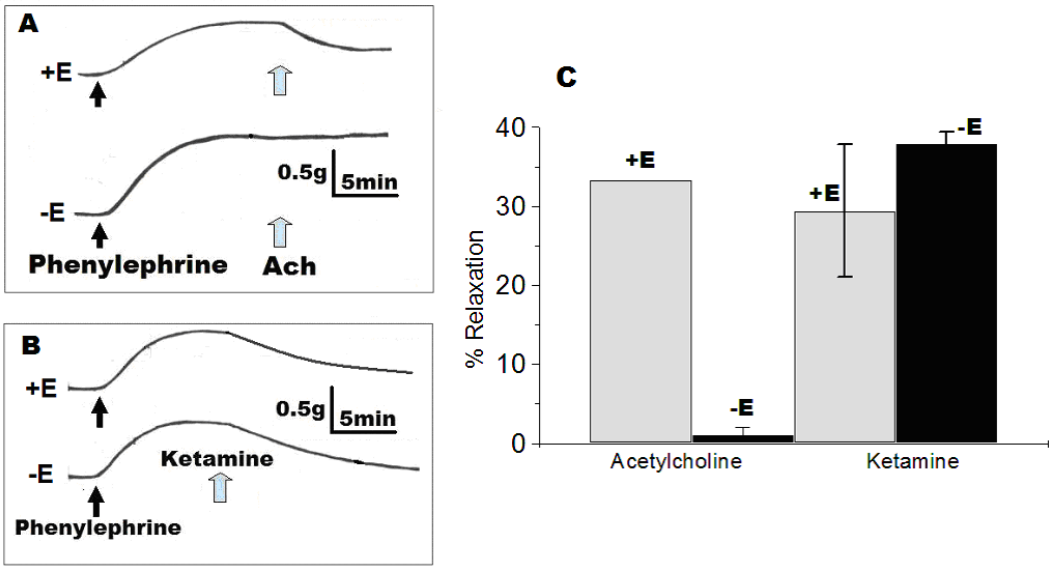

Fig. 3: Typical tracings showing: ***(A)*** *relaxation (upper) and lack of relaxation (lower) responses to acetylcholine (Ach) in aortic rings with intact (+E) or denuded (-E) endothelium and the presence of ketamine relaxation* ***(B)*** *in aortic rings with intact (+E) or denuded (-E) endothelium. The rings were pre-contracted with 10^{-7}M phenylephrine. Summary of experiments in A and B* ***(C)*** *showing that endothelium removal did not significantly influence ketamine-induced relaxation. Means ± SEM; n = 9.*

Discussion

Application of ketamine to unstimulated rabbit aortic rings did not result in a change of baseline tension; suggesting that ketamine has no contractile effect under resting condition. The lack of effect of ketamine on baseline tension is comparable to published reports in the literature that active tone is required to demonstrate the relaxant effect of a variety of vasoactive agents (Bolton, 1979; Ebeigbe and Aloamaka, 1985; Ojeikere et al, 2003).

The results of the present study clearly shows that ketamine induces relaxation of rabbit aortic smooth muscle following pre-contraction induced by phenylephrine or high-K^+. The two contractile agents are known to induce vascular smooth muscle contraction by two separate mechanisms: phenylephrine induces contraction by activating α-adrenergic receptors on the vascular smooth muscle membrane, resulting in Ca^{2+} influx via receptor-operated Ca^{2+} channels (ROCs) as well as Ca^{2+} release from intracellular storage sites. On the other hand, high-K^+ contraction involves membrane depolarization and stimulation of Ca^{2+} influx through potential-sensitive channels (PSCs) (Bolton, 1979; Ebeigbe and Aloamaka, 1987). Our observation that ketamine induces comparable relaxation responses following pre-contraction by both agents suggests that ketamine impairs vascular smooth muscle contraction in a rather non-specific manner: there appears to be no preferential effect of ketamine on mechanisms involving ROCs or PSCs.

We have employed acetylcholine-induced relaxation of phenylephrine pre-contracted rings to assess the integrity of the vascular endothelial cells (Furchgott and Zawadzki, 1980). Our observation that ketamine induced comparable relaxation responses in aortic rings with intact as well as denuded endothelium suggests that the relaxation response is endothelium-independent. One may therefore speculate that impairment of release of nitric oxide or other endothelium-dependent vasorelaxant substance may not be held to account for the observed relaxant effect of ketamine in rabbit aortic smooth muscle.

In conclusion, this study demonstrates that ketamine-induced relaxation of rabbit aortic smooth muscle contraction is endothelium-independent. Further studies aimed at characterizing specific second messenger mechanisms may prove useful.

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