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Enhanced histamine-mediated contraction of rabbit penile dorsal artery in diet-induced hypercholesterolemia

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Abstract

The present study was designed to establish whether penile dorsal arteries isolated from rabbits fed a high cholesterol diet show an enhanced contractile and/or impaired vasodilator response to histamine, and to characterize the histamine receptor subtype involved through in vitro isometric techniques. New Zealand White rabbits were fed a normal diet or a 1% cholesterol diet for 16 weeks. Arteries from cholesterol-fed rabbits retained the ability to relax in response to acetylcholine, whereas histamine and noradrenaline induced a greater contraction response compared to that observed in controls. In both groups, histamine-induced contraction was unaffected by the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), its precursor L-arginine or the cyclooxygenase inhibitor indomethacin. Treatment of arterial rings in the control and hypercholesterolemia groups with the H_1 receptor antagonist, mepyramine, unmasked a vasodilation response to histamine. This was followed by contraction at higher concentrations showing a leftward displacement of the histamine curve compared to controls. The histamine receptor that induced contraction in preparations from the hypercholesterolemic animals was of the H_1 subtype, whereas the receptor involved in histamine-induced relaxation was H_2. The affinity of histamine receptor agonists was comparable to their effects in control animals, and receptor antagonists showed the same potency in both groups. Our findings indicate a preserved endothelial function and enhanced contraction in response to histamine in penile dorsal arteries, probably due to a change in the sensitivity of the contractile machinery of smooth muscle but not a mechanism mediated by a receptor.

Keywords: Rabbit penile dorsal artery; Histamine receptors; Experimental hypercholesterolemia

1. Introduction

Hypercholesterolemia is a factor contributing to erectile dysfunction. Every mmol/l increase in the total cholesterol level is associated with a 1.32 times higher risk of erectile dysfunction (Wei et al., 1994). Improved erectile function by correction of elevated cholesterol levels has been described in men with organic erectile dysfunction (Saltzman et al., 2004). However, no association with total cholesterol was detected in a recent study but it was confirmed that the likelihood of having erectile dysfunction showed an inverse relationship with the level of high density lipoproteins (HDL) (Feldman et al., 1994, 2000; Roumeguère et al., 2003). Findings emerging from studies based on a hypercholesterolemic rabbit model have indicated impaired relaxation of cavernous smooth muscle in response to endothelium mediated and endothelium independent stimuli (Azadzoi and Saenz de Tejada, 1991; Kim et al., 1994). Moreover, it has been recently demonstrated that rats consistently develop erectile dysfunction after being fed a 1% cholesterol diet for 4 months (Bakircioglu et al., 2000). Penile erection is greatly dependent on an adequate inflow of blood to the erectile tissue, and requires coordinated arterial and sinusoidal endothelium-dependent smooth muscle vasodilation caused by a delicate balance between the effects of vasoconstricting and vasorelaxing neurotransmitters/hormones on smooth muscle tone. Accordingly, any factor decreasing the arterial inflow of blood to the corpora may play a role in the pathophysiology of erectile dysfunction.

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There is still much research required to clarify the role of vasoactive factors that affect the structure and function of penile smooth muscle.

Histamine has also been proposed as a possible mediator of penile erection (Adaikan et al., 1991; Andersson and Wagner, 1995). Our recent observations indicate that the effects of histamine on penile vessels from different species (humans, horses and rabbits) is quite variable (Martínez et al., 2000a,b,c, 2002). This amine has been shown to elicit vasodilation, vasoconstriction, or a combination of these responses via stimulation of H_1 receptors, H_2 receptors, or both. Since histamine has long been recognized as an important inflammatory mediator and, the fact that the earliest events in systemic cardiovascular diseases, such as atherogenesis, are of an inflammatory nature (Wick et al., 2004), here we examine the role of this endogenous amine in a experimental model of hypercholesterolemia. This animal model was selected on the grounds of reported similarity between the in vitro reactivity of human and rabbit corpus cavernosum tissue (Azadzoi and Goldstein, 1992).

The present study was designed to test the hypothesis that experimental hypercholesterolemia may enhance contractile and/or reduced relaxant responses to inflammatory mediators such as histamine through differential histamine receptor stimulation in the rabbit penile dorsal artery.

2. Materials and methods

The study procedures and handling of animals were approved by the University Complutense of Madrid (EEC official registration 28079-15ABC). Two groups ($n=20$) of male New Zealand White rabbits (Biocentre S.A., Barcelona, Spain) weighing 2 740±73 g (age 3 months) at the beginning of the study were used. Rabbits were housed identically in individual cages in an air-conditioned room with a 12-h light/darkness cycle. All animals were initially fed a standard laboratory diet (Panlab S.L., Barcelona, Spain) for at least 7 days after delivery to our laboratory. Control rabbits were maintained on a normal diet. To develop high plasma cholesterol levels animals were fed a diet containing 1% cholesterol for 16 weeks (U.A.R. Paris, France). Diet and tap water were available ad libitum. Food intake was monitored daily for the two groups. Blood was collected before sacrifice from the ear vein, and serum lipid profiles were determined with commercially available enzyme kits (BioMerieux, Marcy, France). All animals were anesthetized with intravenous pentobarbital sodium and killed by exsanguination from the common carotid artery. The entire penis was removed and placed in ice-cold physiological salt solution (PSS) to reduce tissue metabolism. Blood supply to the erectile tissues is derived from the penile arteries (dorsal arteries) which are branches of the internal pudendal arteries. The penile dorsal artery (mean external diameter ≈ 250 μm without tension) was identified and dissected free from surrounding connective tissue using a stereomicroscope (Nikon SMZ 2B; Tokyo, Japan). Arterial segments (eight per animal) of approximately 2 mm length were transferred to 5 ml organ baths containing PSS at 37 °C, aireated with 95% O_2 and 5% CO_2 to maintain the pH at 7.4.

2.1. Measurement of isometric tension

The arterial segments were mounted as ring preparations onto parallel stainless steel L-shaped wires (75 μm diameter). One wire was fixed and connected to a displacement unit allowing the fine adjustment of tension. The other was attached to a force transducer (Grass FT03C; Quincy, MA, U.S.A.). Changes in isometric force were recorded on a polygraph (Houston D-5236-5; Austin, Texas, U.S.A.). Preparations were allowed to equilibrate for at least 60 min and washed with fresh PSS at 15 min intervals. Preliminary experiments to calculate the optimal basal tension rendering maximal active contractions were performed by stimulating the arterial rings with a depolarizing potassium solution (K-PSS, 119 mM) until maximal reproducible contractions were achieved. These experiments gave resting tension values around 1 g.

2.2. Measurements of mechanical responses

After the equilibration period, the segments were exposed to K-PSS 119 mM. The integrity of the vascular endothelium was confirmed by immediate relaxation (80–100%) induced by acetylcholine (10^{-6} M) in vessels precontracted with noradrenaline. The relaxant and contractile responses to agonists were evaluated in arterial rings precontracted with noradrenaline ($10^{-6}-4\times10^{-6}$ M) until 50–65% of the K-PSS-induced contraction was achieved. These concentrations produced a stable contraction of sufficient duration to permit the analysis of agonist responses. In experiments exclusively examining contractile ability of agonists, preparations were subjected to the corresponding protocol on basal tension. Cumulative concentration-response curves to histamine receptor agonists were obtained by increasing the organ bath concentration in half log unit steps.

In order to examine the activity of each individual histamine receptor subtype, a given receptor agonist was tested after pretreatment with a specific antagonist to the other two receptor subtypes. To characterize the histamine receptors, the histamine concentration–response curves were performed in the presence of three increasing concentrations of each specific antagonist. It was not possible to reproduce the relaxant effect to obtain several concentration–response curves for the same precontracted arterial segments since tachyphylaxis developed to histamine. Only one dose-response curve could be obtained for each preparation, so it was necessary to use consecutive segments from the same animal in parallel experiments with one ring acting as the control for the other. With respect to the contractile effect on resting tone, previous experiments showed that four concentration-effect curves to histamine, using the same ring, were reproducible. In each experiment, the first concentration–response curve served as a control and the different antagonists were added to the bathing media 30 min before a new concentration–response curve was obtained.