

SILDENAFIL CITRATE (VIAGRA) INDUCED DILATATION OF RESISTANCE VESSELS IN RAT

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ABSTRACT:

Effects of Sildenafil citrate on the perfusion pressure was studied in isolated rat hindquarters *in vitro*, in comparison with H₂-receptor agonist dimaprit and H₂-receptor antagonist cimetidine. These results revealed that Sildenafil citrate at 1x10⁻⁵M and above produced dose related increase in perfusion pressure. Dimaprit at low concentration produced similar response to Sildenafil but was less potent, although high doses of dimaprit produced a mixed response i.e. vasodilatation followed by vasoconstriction. Cimetidine, a potent antagonist of histamine H₂-receptors, significantly (P<0.005) reduced the Sildenafil and dimaprit responses when added in to the perfusion fluid at a concentration of 1x10⁻⁵M. These results suggest that the dilatation of the resistance vessels in isolated perfused rat hindquarter is mediated probably via histamine H₂-receptors. The involvement of histamine H₂-receptors is confirmed by the similarity of the response to dimaprit and the antagonistic response to cimetidine.

INTRODUCTION

Sildenafil citrate (Viagra) is a selective vasodilator that prolongs the action of cyclic guanosine monophosphate (cGMP), the primary mediator of vasodilatation in the corpus cavernosum of the penis, by selectively inhibiting cGMP-specific phosphodiesterase type 5 (PDE5), (Boolel *et al.*, 1996). The wide use of Sildenafil citrate by the patients with erectile dysfunction and cardiovascular disease has resulted in a considerable number of independent studies investigating the cardiovascular safety and functional role of the phosphodiesterase type 5-cyclic guanosine monophosphate-nitric oxide pathway in the cardiovascular system (Conti *et al.*, 1999).

Although there are some data on the hemodynamic effects of Sildenafil, showing slight decreases in blood pressure and no significant change in heart rate, detailed studies of the neural circulatory effects of Sildenafil are lacking. In particular, the effect of Sildenafil on the sympathetic nervous

system, a key contributor to cardiovascular events, is not known.

Sympathetic neural effects of Sildenafil would have direct relevance to understanding any interaction between Sildenafil use and cardiovascular outcome (Gillies *et al.*, 2002).

Further more, Sildenafil has been demonstrated to improve the vasomotor aspect of endothelial dysfunction in patients with heart failure and diabetes (Lepore & Nosari, 2001).

Hemodynamic studies suggest that Sildenafil is a modest vasodilator with the potential to increase coronary blood flow and coronary flow reserve (Zhao *et al.*, 2003).

Although in the patients with ischemic heart disease, Sildenafil is associated with reductions in mean arterial and pulmonary pressure with little effects on systemic or pulmonary vascular resistance (Ghofrani *et al.*, 2002), no effect on cyclic adenosine monophosphate levels in vasculature have been reported. Nevertheless, it is clinically

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important to define clearly the vascular effects of Sildenafil citrate. *In vitro* studies we therefore evaluated the effect of Sildenafil citrate on hemodynamics and vasculature.

MATERIALS AND METHODS

Vascular effects of Sildenafil citrate *in vitro* were investigated using the hindquarters isolated from the rest of the body using ten adult, sexually active male Sprague-Dawley (450-500gm.) rats by established method (Traverse *et al.*, 2000).

Overnight starved rats were administered heparin, 2000 *i.v.* via tail vein. Immediately after the animals were sacrificed, aorta was dissected out free from the surrounded tissues and cannulated just below the renal arteries, to allow perfusion of the hindquarters.

Isolated hindquarters were perfused with modified Krebs-Henseleit buffer solution prepared with distilled deionized (Millipore) water (Peter *et al.*, 2000) at 37°C equilibrated with 5% CO₂ in oxygen, at 5 ml/minute using a pulsative flow pump. Tissue perfusion pressure was measured using research grade displacement transducer model AH 60-2998 at the rate of 35-45 mmHg.

During the measurement of vasodilator activity, perfusion pressure was increased to 130-135 mmHg using phenylephrine (4 x 10⁻⁵M).

Effects of Sildenafil citrate (Pfizer), dimaprit dihydro chloride (Sigma), mepyramine maleate (Sigma) and cimetidine (Sigma) were observed by injecting them into the perfusion fluid in a volume of 0.1 ml at 5 minute intervals with an equilibrium period of at least 15 minutes in case of the introduction of the antagonist. Four dose response curves were thus obtained in each preparation for the antagonist study.

RESULTS

Our results showed that Sildenafil citrate

did not produce any significant effect during the perfusion of the rat hindquarter vasculature at 35-45 mmHg until the concentration of 1x10⁻⁴ M. Above this dose, Sildenafil citrate produced an increase in perfusion pressure associated with vasoconstriction, which remained unchanged by either mepyramine 1x10⁻⁵ M, or cimetidine, 1x10⁻⁵M.

Sildenafil citrate over the concentration of 1x10⁻⁸ to 1x10⁻⁵M produced dose-dependent vasodilatation after the inclusion of phenylephrine (4x10⁻⁵M) in the perfusion fluid. Mepyramine (1x10⁻⁵M) showed no effect on the dose-response curve of Sildenafil citrate, however, cimetidine (1x10⁻⁵M), showed a significant inhibition (P<0.005) of Sildenafil response (Figs.1-A, B, C).

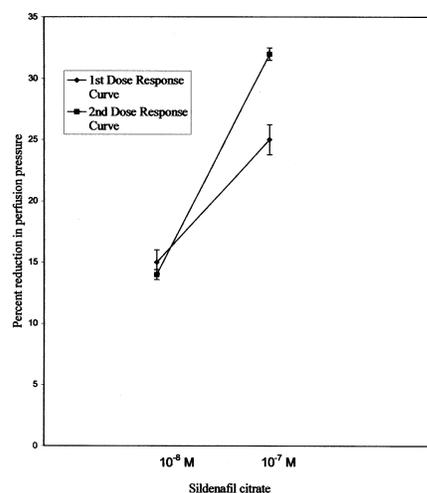


Fig.1a: 1st and 2nd dose-response curve to Sildenafil citrate on rat hindquarters *in vitro* (n=10). Points are mean ± standard errors. n=indicates sample numbers.

Dimaprit also produced similar responses to Sildenafil citrate but was less potent than Sildenafil (Fig. 2A), however at the highest doses (1x10⁻⁴M); vasodilatation followed by vasoconstriction was observed.

A comparison between the dose-response curves of dimaprit alone and in the presence of cimetidine (1x10⁻⁵M, 2x10⁻⁵M and 4x10⁻⁵M),

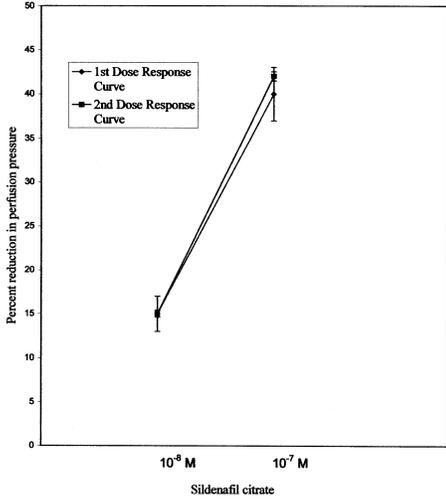


Fig.1b: Effect of Mepyramine ($1 \times 10^{-5} M$) on 1st and 2nd dose-response curve to Sildenafil citrate on rat hindquarters *in vitro* (n=10). Points are mean \pm standard errors. n=indicates sample numbers.

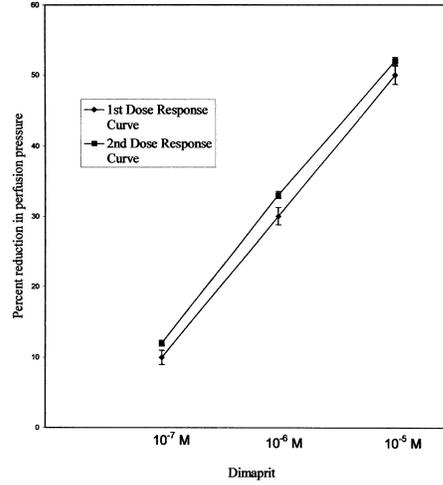


Fig.2a: 1st and 2nd dose-response curve to Dimaprit on rat hindquarters *in vitro* (n=10). Points are mean \pm standard errors. n=indicates sample numbers.

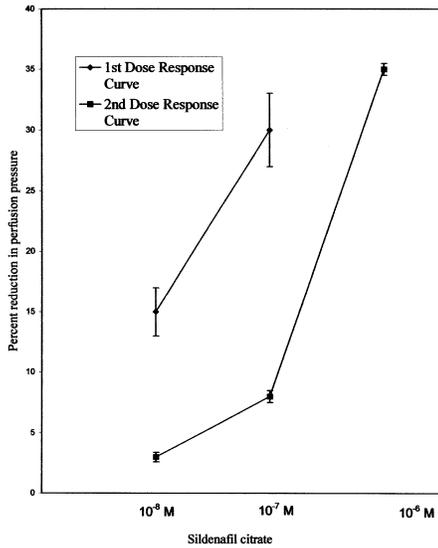


Fig.1c: Effect of Cimetidine ($1 \times 10^{-5} M$) on 1st and 2nd dose-response curve to Sildenafil citrate on rat hindquarters *in vitro* (n=10). Points are mean \pm standard errors. n=indicates sample numbers.

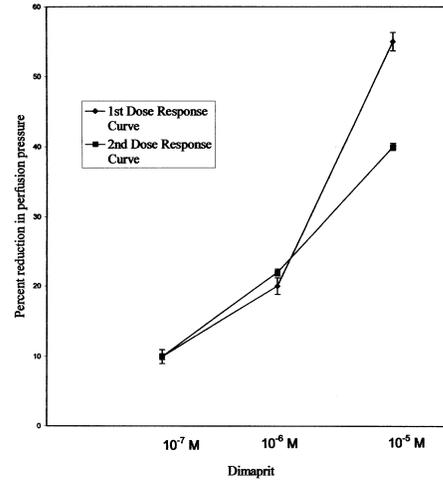


Fig.2b: 1st (Control) and 2nd (Cimetidine $1 \times 10^{-5} M$) dose-response curve to Dimaprit on rat hindquarters *in vitro* (n=10). Points are mean \pm standard errors. n=indicates sample numbers.

showed that cimetidine caused concentration dependent inhibition of the response to dimaprit (Figs. 2-B, C, D).

Dose-response curves to dimaprit in the absence of cimetidine and in the presence of cimetidine ($1 \times 10^{-5} M$ and $3 \times 10^{-5} M$) are presented in Fig.3. These results indicated that the inhibitory effect of cimetidine to the response

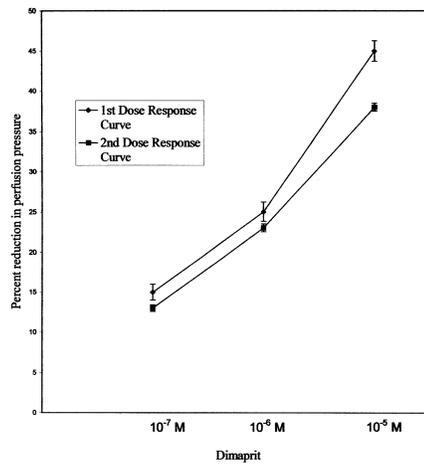


Fig.2c: 1st(Control) and 2nd(Cimetidine 2x10⁻⁵M) dose-response curve to Dimaprit on rat hindquarters *in vitro* (n=10). Points are mean \pm standard errors. n=indicates sample numbers.

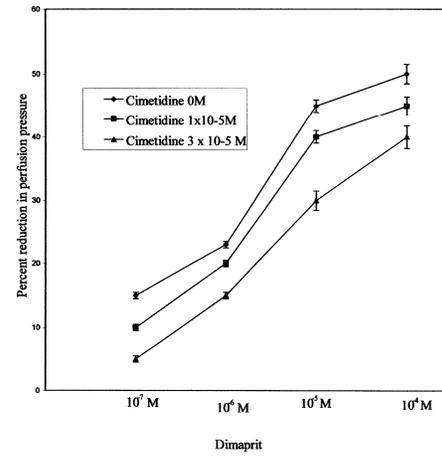


Fig.3: Dose-response curve to dimaprit-induced vasodilatation in the absence of cimetidine and in the presence of different concentrations of cimetidine in rat hindquarters *in vitro* (n=10). Points are mean \pm standard errors. n=indicates sample numbers.

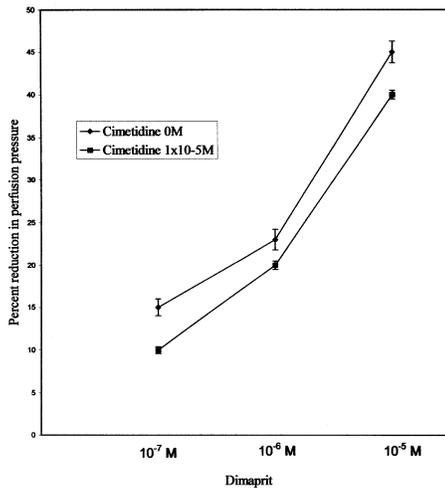


Fig.2d: 1st(Control) and 2nd(Cimetidine 4x10⁻⁵M) dose-response curve to Dimaprit on rat hindquarters *in vitro* (n=10). Points are mean \pm standard errors. n=indicates sample numbers.

of dimaprit was dose dependent and that the dose-response curves to dimaprit were displaced by the effect of cimetidine.

DISCUSSION

Levels of cGMP in vascular smooth muscles are tightly regulated by several cyclic nucleotide phosphodiesterase enzymes (PDEs) that catalyze cGMP degradation and terminate this second messenger signal (Stief *et al.*, 2000).

Sildenafil citrate (Viagra, Pfizer), is a highly selective inhibitor of PDE5 that potentiates the activity of cGMP in the corpus cavernosum, thereby augmenting vasodilator activity of neuronally mediated nitric oxide production (Michelakis *et al.*, 2002).

Sildenafil has also been demonstrated to increase the cGMP levels and cause smooth muscle relaxation in isolated segments of epicardial coronary artery. However, the effect of Sildenafil citrate on the coronary resistance vessels has not been studied extensively.

The present study has been undertaken to investigate whether selective inhibition of PDE5 with Sildenafil results in the involvement of histaminergic H₂ receptors in

maintaining the perfusion pressure of the rat hindquarters.

Our results showed a dose-dependent dilatation of resistant vessels by Sildenafil citrate (an increase in the perfusion pressure), at a concentration of 10^{-4} M and above in 8/10 (80%) preparations, thus suggesting a modest vasodilator effect on the resistance vessels *in vitro*. Dimaprit, like Sildenafil citrate, also caused about 10% less potent but similar effect in 7/10 (70%) preparations. These results indicate that the rat hindquarters responded to Sildenafil citrate that increases the perfusion pressure with sensitivity comparable to that for dimaprit.

Our results further showed that after the inclusion of phenylephrine in the perfusion fluid, the responses of Sildenafil were unaltered by Mepyramine, but were significantly reduced by cimetidine. Cimetidine also caused concentration dependent inhibition of the responses to dimaprit.

In vivo studies on rat hindquarters have demonstrated the presence of both histamine H_1 and H_2 -receptors and the interaction between histamine and histamine antagonists is similar to that which occurs in most other vascular beds (Sugiyama *et al.*, 2001).

In animal studies, Sildenafil citrate seems to produce large changes in cardiac function in many animal species both *in vivo* and *in vitro*. The cardiac response to Sildenafil in the guinea-pig *in vitro* induces increases in sinus rate, ventricular contraction; coronary flow and external pressure volume work (Geelen *et al.*, 2000).

The above cardiac actions are all competitively antagonized by histamine H_3 -receptor antagonists and therefore involve stimulation of histamine H_3 -receptors (Salata *et al.*, 1995); little information is, however, documented regarding the stimulation of H_2 -receptors. In our results, selective antagonism of Sildenafil responses by cimetidine indicates the involvement of H_2 -receptors, which was

further confirmed by the similarity of the response to dimaprit.

Despite the clear evidence for H_1 and H_2 -receptors associated with dilatation of rat hindquarters resistance vessels *in vivo*, the present studies thus confirms the involvement of H_2 -receptors, since the responses to Sildenafil were inhibited by cimetidine, a competitive histamine H_2 -receptor antagonist in many *in vitro* biological systems. The reasons for the loss of H_1 -receptor vasodilatation *in vitro* has not been clarified in the present studies, since, dimaprit, like Sildenafil citrate, elicited the dose-dependent dilatation, where as mepyramine was relatively ineffective.

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