

## **ROLE OF H<sub>2</sub> RECEPTORS ON THE CARDIAC ACTION OF SILDENAFIL CITRATE (VIAGRA) IN PERFUSED RAT HEART**

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### **ABSTRACT**

The present study deals with the role of H<sub>2</sub>-receptors on the cardiac action of Sildenafil citrate (Viagra) using histamine H<sub>2</sub>-receptor antagonist cimetidine in mammalian isolated heart. Effects of Sildenafil citrate on cardiac force, heart rate, and coronary perfusion pressure were studied in isolated perfused rat heart in comparison with H<sub>2</sub>-receptor agonist dimaprit and H<sub>2</sub>-receptor antagonist cimetidine. These studies revealed that Sildenafil at 10<sup>-6</sup> M and above produced dose related increases in contractile force and the heart rate. The maximum observed increase at 10<sup>-7</sup> M represented a 40.4% ± 6.1% increase above control amplitude. A similar dose-related decrease in coronary perfusion pressure was also observed at the same time. Dimaprit at a concentration of 10<sup>-5</sup> M produced similar responses to the Sildenafil but was less potent. Cimetidine, a potent antagonist of histamine H<sub>2</sub>-receptors, competitively inhibited the positive inotropic effect of Sildenafil when added in to the perfusion fluid at a concentration of 10<sup>-7</sup> to 10<sup>-5</sup> M. These results indicated that rat heart responds to Sildenafil citrate, which increases contractile force and enhances heart rate, with sensitivity comparable to that for dimaprit. The selective antagonism of the Sildenafil contractility response by cimetidine indicated that this response is mediated via histamine receptors. The involvement of histamine H<sub>2</sub>-receptors is confirmed by the similarity of the response to dimaprit. It is thus concluded that these effects of Sildenafil citrate in the isolated perfused rat heart are mediated via modified histamine H<sub>2</sub>-receptors.

### **INTRODUCTION**

As a potent and selective inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase-5 (PDE5), Sildenafil citrate (Viagra) has been approved as the first oral medication for the treatment of erectile dysfunction in men of diverse etiologies, including patients with common cardiovascular diseases who are not receiving organic nitrates or nitrate donor drugs (Gillies et al 2002; Jackson 2001).

The wide use of Sildenafil citrate by patients with erectile dysfunction and cardiovascular disease has resulted in a considerable number of independent studies investigating the

cardiovascular safety and the functional role of the (PDE5) nitric oxide pathway in the cardiovascular system (Jiang et al 2002; Kloner 2000; Conti *et al.*, 1999).

In hemodynamic studies, Sildenafil has been found to produce small decrease in systemic and pulmonary blood pressure but caused no adverse cardiovascular effects in specific populations of men with coronary heart disease. Sildenafil citrate has been found a modest vasodilator with the potential to increase coronary blood flow and coronary flow reserve in the patients with heart failure and diabetes. In the patients with ischemic heart disease, Sildenafil is associated with reduction in mean arterial and pulmonary

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pressure with little effect on heart rate, cardiac output, and systemic or pulmonary vascular resistance (Watanabe et al 2002; Michelakis et al 2002).

Sildenafil has also been found to cause a positive effect on coronary flow reserve in men with severe coronary artery disease. Together with the findings with other studies, these data suggests that PDE5 may play an important role in the regulation of coronary blood flow in the healthy and diseased heart (Hoeper et al 2002; Herrmann et al 2000).

Sildenafil has been demonstrated to increase cGMP levels and cause smooth muscle relaxation in isolated segments of epicardial coronary artery. However, the effects of Sildenafil on coronary resistance vessels have not been studied. Similarly the role of Sildenafil citrate in the regulation of the coronary circulation and the nature of the coronary histamine receptors are not well studied (Boolell et al 1996).

Consequently, the present study has been undertaken to investigate the action of Sildenafil citrate in the regulation of perfusion pressure in the coronary vessels and heart contractility and to elucidate the role of H<sub>2</sub> receptors on cardiac action of Sildenafil using H<sub>2</sub> receptor antagonist.

## **MATERIALS AND METHODS**

Positive inotropic effects of the Sildenafil citrate on the rat were investigated using Langendorff perfused heart preparations obtained from ten adult, sexually active male Sprague-Dawley (450-500gm.) rats by established methods (Drolet et al 1998). Immediately after excision, the samples were placed in modified Krebs-Henseleit buffer solution prepared with distilled deionized (Millipore) water (Peter et al 2000) at 4°C, then transferred to solution equilibrated with 5% CO<sub>2</sub> in O<sub>2</sub> at ambient temperature. The heart was perfused through a cannula inserted into the ascending aorta, with the modified Krebs-Henseleit buffer solution equilibrated with 5%

CO<sub>2</sub> in O<sub>2</sub> at 37.5°C. Krebs solution was perfused by a peristaltic pump at a rate of 14-17 ml/minute. This procedure induced an initial perfusion pressure of 14-25 mmHg, which gradually increased to 30-45 mmHg within 45 minutes and was kept constant throughout the experiment.

Coronary perfusion pressure was recorded with a Statham pressure transducer. Apex of the heart was mounted vertically in a 60 ml Harvard single tissue glass organ bath containing modified Krebs-Henseleit buffer solution equilibrated with 5% CO<sub>2</sub> in O<sub>2</sub> at 37.5°C. The lower end of the preparation was connected to a hook provided with a stainless steel rod, while the other end was connected to the research grade Harvard isometric displacement transducer model (AH 60-2997) by means of strong cotton thread. This tissue which normally did not exhibit spontaneous contraction was placed under 1 g. resting tension and isometrically stimulated to contract with a pulse of supramaximal voltage (usually 20-30 V) and of 2mS duration using a Grass stimulator (Model S88, USA). Contractile force and coronary perfusion pressure were recorded on a Harvard 2-channel modular Universal Oscillograph through amplifier interface model (AH 50-8861).

The preparations were allowed sufficient time to develop a stable contraction (approximately 45 minutes) during which the bath fluid was continuously over flowed. Injections of Sildenafil citrate were made into the perfusion circuit proximal to the cannulated part of aorta and the dose response curve of Sildenafil was determined on perfusion pressure, contractile force and heart rate, before and after adding the antagonists into the perfusion fluid.

The effects of the Sildenafil citrate on contractility (tension amplitude) were compared with the selective histamine H<sub>2</sub> receptor agonist dimaprit by obtaining cumulative dose response curves to the agonist. Drug solutions were added to the bath in a volume not exceeding 0.1 ml and responses allowed to reach a stable maximum before

**Table-1**

Increase of heart rate (% of control) after single injections of Sildenafil citrate through the coronary artery of isolated perfused rat heart without cimetidine added in to the perfusion medium

| Dose of Sildenafil Citrate | % Increase of Heart Rate<br>(Mean $\pm$ S.E.M.)<br>(Without cimetidine) |
|----------------------------|-------------------------------------------------------------------------|
| $4.5 \times 10^{-7}$       | 18.5 $\pm$ 4.5 (n=10)                                                   |
| $8.5 \times 10^{-7}$       | 24.5 $\pm$ 6.2 (n=10)                                                   |
| $1.5 \times 10^{-6}$       | 48.5 $\pm$ 9.7 (n=10)                                                   |
| $3.5 \times 10^{-6}$       | -                                                                       |
| $6.5 \times 10^{-6}$       | -                                                                       |
| $1.5 \times 10^{-5}$       | -                                                                       |
| $2.5 \times 10^{-5}$       | -                                                                       |

n= total number of samples examined.

**Table-2**

Inhibition in the increase of heart rate (% of control-compared with the results without cimetidine-table-1) by cimetidine added in to the perfusion medium after the single injections of Sildenafil citrate through the coronary artery of the isolated perfused rat heart

| Dose of Sildenafil Citrate | Change of Heart Rate<br>(Mean $\pm$ S.E.M.)<br>(With cimetidine $10^{-5}$ M) | Change of Heart Rate<br>(Mean $\pm$ S.E.M.)<br>(With cimetidine $10^{-4}$ M) |
|----------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| $4.5 \times 10^{-7}$       | -                                                                            | -                                                                            |
| $8.5 \times 10^{-7}$       | 10.0 $\pm$ 0.2 (n=10)                                                        | -                                                                            |
| $1.5 \times 10^{-6}$       | 15.5 $\pm$ 2.9 (n=10)                                                        | -                                                                            |
| $3.5 \times 10^{-6}$       | 25.4 $\pm$ 4.1 (n=10)                                                        | -                                                                            |
| $6.5 \times 10^{-6}$       | 36.7 $\pm$ 4.6 (n=10)                                                        | 15.6 $\pm$ 4.2 (n=10)                                                        |
| $1.5 \times 10^{-5}$       | -                                                                            | 30.5 $\pm$ 5.2 (n=10)                                                        |
| $2.5 \times 10^{-5}$       | -                                                                            | 15.6 $\pm$ 4.2 (n=10)                                                        |

n= total number of samples examined.

increasing the bath concentration. After completion of dose response curves tissues were washed by overflow and allowed 15-30 minutes to recover.

The effects of histamine receptor antagonists cimetidine (H<sub>2</sub>), on the Sildenafil citrate responses were also investigated. For this study control dose response curves to Sildenafil in random order were first obtained in each preparation. The antagonist was then

added to the perfusion medium and an equilibration period of at least 15 minutes allowed during which the bath fluid was changed at least every 5 minutes. Sildenafil dose response curves were then repeated in the same order as that before antagonist administration.

The results were statistically evaluated by Student's *t*-test.

Drugs used were Sildenafil citrate (Pfizer), cimetidine (Sigma), and dimaprit-dihydro chloride (Sigma).

### RESULTS

Sildenafil at  $10^{-6}$  M and above produced dose related increases in contractile force and

the heart rate and a decrease in the coronary perfusion pressure. The maximum observed increases at  $10^{-4}$  M represented a  $44.4\% \pm 6.1\%$  increase above control amplitude (n=10).

Dimaprit when added into the perfusion medium at a concentration of  $10^{-5}$  M did not influence the effects of Sildenafil on heart rate,

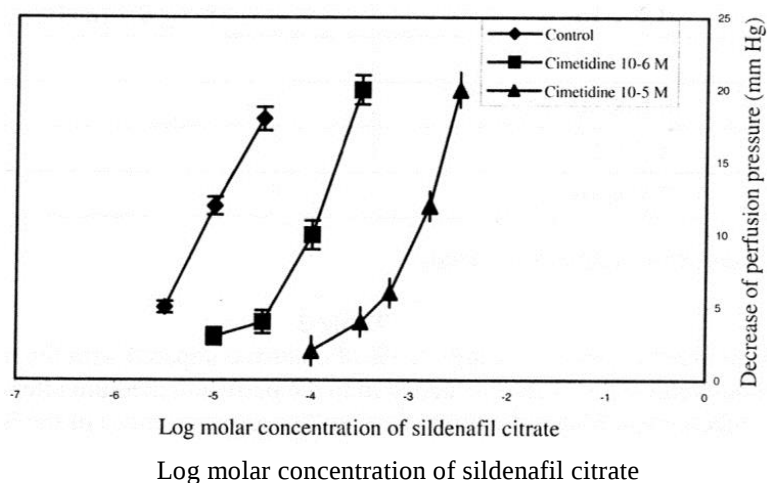


Figure-1: Log dose-response curve of sildenafil citrate for coronary perfusion pressure. Each point represents mean of ten experiments. Vertical bars indicates S.E.M.

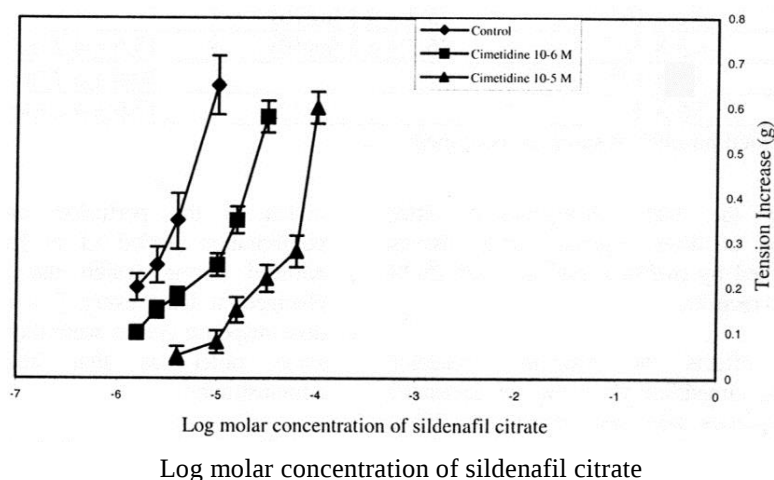


Figure-2: Log dose-response curve of sildenafil citrate for heart contractility. Each point represents mean values of ten experiments. Vertical bars indicates S.E.M.

heart contractility and on perfusion pressure of coronary vessels.

The Sildenafil dose-response curve repeated in the presence of cimetidine  $10^{-7}$  M to  $10^{-5}$  M lay to the right of control indicating antagonism by cimetidine of the Sildenafil response. Cimetidine inhibited the positive inotropic effect of Sildenafil citrate on heart muscle and the depressor effect of the Sildenafil and dimaprit on the coronary perfusion pressure. The minimum effective dose of the cimetidine was  $10^{-7}$  M (Figures.1&2).

Results for the increase of heart rate (% of control), after the single injections of Sildenafil citrate through the coronary artery of the isolated perfused rat heart and its inhibition by cimetidine added into the perfused medium is presented in tables 1&2. Addition of cimetidine  $10^{-5}$  M to  $10^{-4}$  M into the perfusion medium inhibited the positive inotropic effects of Sildenafil, however, cimetidine, itself did not change the heart rate when given by the single injection or when added into the perfused medium.

## DISCUSSION

Sildenafil has been shown to be effective in men with hypertension, diabetes, non-vascular organic etiologies for erectile dysfunctions, and psychogenic causes (Vitezic et al 2001). Although men with chronic stable ischemic heart disease responded to Sildenafil, the drug has not been tested in the patients with severe or acute cardiovascular disease such as patients with unstable angina or recent (within 6 months) strokes or life-threatening arrhythmia. The drug has also not been studied in a systematic fashion in men with congestive heart failure (Boyce and Umland 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, force of ventricular contraction, coronary

flow, aortic flow, total cardiac output, and external pressure-volume work (Geelen *et al.*, 2000). The above cardiac actions of Sildenafil are all competitively antagonized by histamine  $H_3$ -receptor antagonists and therefore involve stimulation of histamine  $H_3$ -receptors (Ekins *et al.*, 2002; Salata *et al.*, 1995), little information is however documented regarding the stimulation of histamine  $H_2$  receptors (Swissa *et al.*, 2002). In the present study we have therefore investigated the histaminergic  $H_2$  stimulatory effects of Sildenafil citrate (Viagra), and dimaprit on rat right atrial muscles *in vitro*.

Our results showed a dose dependent positive inotropic effect and decreased perfusion pressure produced by Sildenafil citrate, and dimaprit. The spontaneous rate was not quantified; however, the incidence of spontaneous activity, and the drug concentration at which it first occurred was noted. Sildenafil up to  $10^{-4}$  M produced spontaneous activity in 8/10 (80%) preparations. Activity was first noted at  $10^{-6}$  M in four preparations (40%) and by  $10^{-5}$  M spontaneous activity occurred in 5/10 (50%) preparations. Dimaprit up to  $10^{-4}$  M produced spontaneous activity in 7/10 (70%) preparations. These results indicate that the rat heart responded to Sildenafil citrate that increases contractility and enhances the heart rate, with sensitivity comparable to that for dimaprit.

The selective antagonism of the histaminergic contractility response by cimetidine indicates that this response is mediated via histamine  $H_2$ -receptors. The antagonism between Sildenafil citrate and cimetidine seems to be competitive since the log dose-response curves of Sildenafil in all parameters investigated were shifted to the right but remained parallel to the control. The involvement of  $H_2$ -histamine receptor was confirmed by the similarity of the response to dimaprit. It is thus concluded that Sildenafil citrate (Viagra) induced positive inotropic effects in isolated perfused rat heart are probably mediated via histamine  $H_2$ -receptors.

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