THE EFFECTS OF DIFFERENT DOSES OF VARDENAFIL ON CORONARY AUTO-REGULATION IN ISOLATED RAT HEART

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ABSTRACT

Phospodiesterase-5 (PDE5) catalyses cyclic GMP (cGMP) degradation and regulates its intracellular content. Specific inhibition of that isoenzyme induces an increase in cGMP concentration, leading to smooth muscle cell relaxation and consequent vasodilatation. With regard to this information, the aim of our study was to compare the effects of different PDE5 inhibitors on coronary blood flow and the L-arginine/NO system in isolated rat hearts. Hearts were isolated from male Wistar albino rats (n=12 rats) and perfused with buffer at a constant pressure. Coronary auto-regulation (CA) was investigated with follow-up of coronary perfusion pressure (CPP) changes from 40 to 120 cm H2O. After the first sequence of CPP changes (basic protocol), the hearts were perfused with vardenafil in different doses (10, 20, 50, 200 nM) alone or in combination with nitric oxide synthase inhibitor (L-NAME, 30 μM). During control conditions the hearts exhibited CA between 50 and 90 cm H2O, with a basal coronary flow (at 60 cm H2O) of 6.63±0.30 ml/min. Vardenafil induced significant vasodilatation at a dose of 200 nM (about 20% at all CPP-values) but not at other applied doses. Additional application of L-NAME induced decreases in coronary flow (CF) in all treated groups. Nevertheless, those effects were significant only at the lowest and highest doses of vardenafil.

Key words: PDE5 inhibitors, coronary circulation, NO

SAŽETAK

Fosfodiesteraza 5 katalizira razgradnju cikličnog GMP utičući na njegovu intračelijsku koncentraciju. Specifična inhibicija tog izoenzima indukuje povećanje koncentracije cikličnog GMP, dovodeći do relaksacije glatkih mišićnih ćelija i posledične vazodilatacije. U skladu sa tim, cilj naše studije je bio da uporedimo efekte različitih inhibitora PDE5 na koronarni protok i L-arginin/NO sistem izolovanog srca pacova. Srca, izolovana iz pacova Wistar albino soja (muškog pola, n=12) su perfudovana vardenafilm (10, 20, 50, 200 nM), kao i istim dozama vardenafila u kombinaciji sa inhibitorom NO sintaze (L-NAME, 30 μM). U kontrolnim CA se odvijala izmedju 50 i 90 cm H2O, pri bazalnim vrednostima protoka (60 cm H2O) od 6.63±0.30 ml/min. Vardenafil indukuje sniženje koronarnog protoka u dozi od 200 nM (oko 20% na svim CPP-vrednostima), ali ne i pri ostalim apškriptivnim dozmama. Dodatna administracija L-NAME-a indukuje sniženje koronarnog protoka u svim tretiranim grupama. Ipak, samo pri najmanjim i najvišim vrednostima Vardenafila ovi efekti su bili signifi kantni.

Naša saznanja jasno pokazuju da svi ispitivani inhibitori PDE5 ostvaruju uticaj na koronarnu auto-regulaciju posredstvom L-arginin/NO sistema.

Key words: inhibitor PDE5 - koronarna cirkulacija - NO


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INTRODUCTION

Cyclic nucleotides (Cyclic adenosine 3’5’-monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP)) function as major intracellular secondary messengers to mediate biological responses initiated by diverse extracellular signals (1). Cyclic nucleotide phosphodiesterases (PDEs) catalyse cAMP and cGMP and are important determinants regulating the intracellular concentrations and biological actions of both of these secondary messengers. Twelve families of these enzymes have been described so far (2).

Endothelial cells express three PDE isoforms: PDE2, PDE4 and PDE5, the last of which is a cGMP-specific PDE. This isoform is of special interest for our investigation regarding involvement in the nitric oxide (NO)/cGMP signalling pathway (3). Specifically, modulation of PDE5 activity directly influences all NO-mediated effects via the NO/cGMP cascade. Highly selective PDE5 inhibitors significantly amplified NO-mediated vascular effects in experimental (2) and clinical trials (4, 5). The most interesting clinical topic in a last few years is usage of a specific PDE5 inhibitor, sildenafil (Viagra®), in treatment of erectile dysfunction (6). PDE5 inhibitors cause vasorelaxation in various vascular beds by increasing intracellular cGMP levels in vascular smooth muscle (7). Hence, their intake is commonly associated with a moderate reduction in systolic and diastolic blood pressure in humans (5). Sildenafil has additional effects that are external to the corpus cavernosum, particularly strong vasodilatory effects highly selectively for pulmonary circulation. Treatment of pulmonary hypertension with sildenafil showed beneficial effects that were amplified by the addition of inhaled NO through interference with the L-arginine/NO/cGMP system at distinct targets, thereby potentiating their actions (6, 8).

Recent studies have indicated that PDE5 inhibitors such as sildenafil, vardenafil or tadalafil induce preconditioning-like effects in the heart and protect against ischemia/reperfusion injury (9,10), leading to accumulated cGMP. The cGMP/PKG pathway has been shown in many reports to be involved in the protective signalling of preconditioning. Direct PKG activation with a cGMP analogue has proven to be protective (11), while receptor-mediated preconditioning could be blocked with a GC inhibitor (12). Salloum et al. (2007) used in situ rabbit hearts to demonstrate that sildenafil and vardenafil limited myocardial infarction when administered at the time of reperfusion in a model of ischemia/reperfusion by a mechanism that involved MKATP. Additionally, in a similar model performed on isolated rat hearts, du Toit et al. (2005) showed that low concentrations of sildenafil (20-50 nM) improve reperfusion function while higher concentrations (200 nM) worsen it.

For this purpose, the aim of our study was to evaluate the effects of a novel PDE5 inhibitor, vardenafil, on coronary auto-regulation in the isolated rat heart as one kind of ischemia/reperfusion model, with possible impact on the endothelial L-arginine/NO system.

MATERIAL AND METHODS

Isolated rat heart preparation

The hearts (n=12) excised from Wistar albino rats, male sex, body mass of about 200 g (obtained from Military Technical Institute, Belgrade, Serbia and Montenegro) were perfused with a Langendorff apparatus (Hugo Sachs Elektronik-Harvard Aparatus GmbH, March-Hugstetten, Germany). After short-term ether narcosis, the animals were killed by cervical dislocation (Schedule 1 of the Animals/Scientific Procedures, Act 1986, UK), with heparin premedication as an anticoagulant. After urgent thoracotomy and rapid heart arrest by superfusion with ice-cold isotonic saline, the hearts were rapidly excised and isolated. The aortas were cannulated and retrograde perfused according to the technique for constant pressure conditions. The composition of the non-recirculating Krebs-Henseleit perfusate was as follows (mmol/l): NaCl 118, KCl 4.7, CaCl2 x 2H2O 2.5, MgSO4 x 7H2O 1.7, NaHCO3 25, KH2PO4 1.2, glucose 11, pyruvate 2, equilibrated with 95% O2 plus 5% CO2 and warmed to 37°C (pH 7.4). All hearts were electrically paced (5 V, 320 bpm) by the electronic stimulator (Hugo Sachs Elektronik-Harvard Aparatus GmbH) and constant left ventricular drainage through the dissected mitral valve was performed.

Physiological assay

After the heart perfusion had been set up, a 30-minute period was allowed for stabilisation of the preparation. During the stabilisation period, all hearts were challenged by short-term occlusions (5-30 seconds) as well as by bolus injection of 5 mmol/l adenosine (60 µl at a flow rate of 10 ml/min) to elicit maximal coronary flow. The hearts were discarded (about 25%) if the flow did not increase by 100% over the control value (for both tests). After the equilibration period, coronary perfusion pressure was lowered to 50 and 40 cm H2O and then gradually increased to 70, 80, 90, 100, 110 and 120 cm H2O to establish coronary auto-regulation. When the flow was considered to be stable at each value of perfusion pressure, samples of the coronary effluent were collected. Properly performed control experiments were included in the study (i.e., the groups of the hearts in which the coronary perfusion pressure/coronary flow relationship were studied twice in the absence of any drug). It was essential to confirm that the preparation used was stable and that the responses to the first and second run of changes in perfusion pressure did not differ substantially. In the control protocol, preparation stabilisation was performed at a basal coronary perfusion pressure of 60 cm H2O for 30 minutes.

Experimental protocols

In the experimental protocol hearts were perfused with or without vardenafil (a specific PDE5 inhibitor) at different doses: 10, 20, 50 and 200 nM. In a second experimental protocol hearts were perfused with vardenafil (10, 20, 50 and 200 nM) plus an inhibitor of nitric oxide synthesis, Nω-nitro-L-arginine monomethyl ester (L-NAME, 30 µM) (3).
Statistical analysis

Values are expressed as means ± standard error (SE). Statistical analysis was performed by multifactorial analysis of variance for repeated measurements between subject factors as well as the Bonferroni test. P values less than 0.05 were considered to be significant.

RESULTS

Control conditions

The results showed that isolated rat heart auto-regulated between 50 and 90 cm H2O, which was reported previously (13) and slightly different in comparison to the auto-regulatory range of the isolated guinea pig heart (14). Coronary flow in the auto-regulatory range varied from 4.97±0.22 ml/min/g wt at 50 cm H2O to 6.68±0.25 ml/min/g wt at 90 cm H2O (Fig. 1-4).

PDE5 inhibition and coronary flow vs. PDE5+NOS inhibition and coronary flow

Perfusion with different concentrations of vardenafil induced a non-significant increase in coronary flow from 40 to 120 cm H2O (Fig 1A-3A), except in the high-dose group (200 nM) (Fig 4A). Additional NOS inhibition with L-NAME reversed vardenafil-induced effects on coronary flow (Fig 1B-4B), with statistical significance seen only at the lowest (10 nM) and highest (200 nM) concentrations of Vardenafil.

DISCUSSION

The present study showed that administering vardenafil does not significantly influence coronary flow in an isolated rat heart, except at the high dose of 200 nM. At all lower concentrations (10, 20, 50 nM) vardenafil did not influence coronary circulation. That result is in accordance with previous studies (10), with no clear explanation. That effect could be dependent on the activity of GC and the cGMP-dependent kinase (PKG).

Vardenafil and other PDE-5 inhibitors are established drugs in the treatment of erectile dysfunction in men. PDE enzymes hydrolyse the phosphodiester bond of the cyclic nucleotides cAMP and cGMP, which serve as second messengers in various cellular functions. Therefore, PDE inhibition can elevate intracellular cAMP or cGMP concentration, depending on their substrate specificity. Type-5 PDEs predominantly metabolise cGMP and are localised in many tissues, including canine and mouse ventricular myocytes (15, 16, 17). In the heart, increasing intracellular cGMP via the addition of a cell-permeable cGMP analogue leads to reduced infarct size after ischemia/reperfusion with either pre-treatment (11) or treatment at reperfusion (18). Therefore, it seemed only logical that indirect cGMP elevation via PDE-5 inhibition would show similar effects. Ockaili (2002) was the first to show that sildenafil administered before ischemia reduced myocardial infarct size in an in situ rabbit heart model (19). Later, similar results could be shown for other PDE-5 inhibitors, such as vardenafil and tadalafil, in various models (20). Du Toit (2005) confirmed these results regarding sildenafil at the lowest concentration but not at the highest concentration, which was the basic postulate for our study (21).

However, PDE-5 inhibitors have proved to be effective not only when given before ischemia as preconditioning agents, but also when applied at reperfusion as post-conditioning agents. Salloum (2007) reported a marked reduction in infarct size in rabbit hearts in situ when sildenafil or vardenafil was administered at reperfusion (22). In the present study, we confirmed these data for vardenafil in isolated rat hearts with approximately the same concentration of the drug used in the earlier study. In our hands, administration of 10, 20 and 50 nM vardenafil had no sig-

![Fig. 1: Effects of A) 10 nM vardenafil or B) 10 nM vardenafil +30 μM L-NAME on coronary flow in isolated rat hearts compared to the control (n=6). The values are expressed as mean ± S.E.M., ** p< 0.01.](image)
Fig. 2: Effects of A) 20 nM vardenafil or B) 20 nM vardenafil + 30 μM L-NAME on coronary flow in isolated rat hearts compared to the control (n=6). The values are expressed as mean ± S.E.M., ** p< 0.01.

Fig. 3: Effects of A) 50 nM vardenafil or B) 50 nM vardenafil + 30 μM L-NAME on coronary flow in isolated rat hearts compared to the control (n=6). The values are expressed as mean ± S.E.M., ** p< 0.01.

Fig. 4: Effects of A) 200 nM vardenafil or B) 200 nM vardenafil + 30 μM L-NAME on coronary flow in isolated rat hearts compared to the control (n=6). The values are expressed as mean ± S.E.M., ** p< 0.01.
significant effect on the coronary vascular bed, while the high dose of 200 nM induced significant vasodilatation (Fig 1A-4A). This somewhat surprising result was in agreement with a report from du Toit et al. (2005), wherein they observed clear infarct size reduction with a pre-treatment of 50 nM sildenafil, but increasing the concentration to 200 nM removed this protective effect (21). Our results are also in accordance with a recent study on treated infarct size in isolated rat hearts (10).

Additional inhibition of the L-arginine/NO system reduced coronary flow in the presence of 10 and 200 nM vardenafil, but not when 20 and 50 nM were applied (Fig 1B-4B). That aspect of our results suggests that only protective and high doses of vardenafil might influence the coronary system via the NO system. Regarding to their combined intake with NO-liberating drugs such as organic nitrates (sublingual nitroglycerin, isosorbide dinitrate/mononitrate), this is absolutely contraindicated because such combinations may lead to excessive and uncontrolled hypotension (23) and these results shed new light on that problem. These deleterious interactions may be explained by the fact that nitrates (by leading to enhanced NO production and a rise in cGMP levels) and sildenafil (by inhibiting the decay of cGMP via PDE5) both interfere with the same system at distinct targets, thereby mutually potentiating their actions. Hence, the distinct interactional properties of NO-liberating drugs with PDE5 inhibitors may depend on the oxidative or anti-oxidative properties of the respective drug (7).

Our results suggest that the lowest (10 nM) and highest (200 nM) doses of vardenafil act via the NO system. Further measurement of oxidative stress parameters in that experimental model could indicate which dose of vardenafil is protective with regard to anti-oxidative potential.

REFERENCES


