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Relaxant effect of sildenafil in the rabbit basilar artery

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Abstract

We hypothesized that sildenafil, inhibitor of phosphodiesterase-5 (PDE-5), interacts with the nitric oxide (NO)-cGMP pathway in the cerebral arteries and shows vasoactive effects. To prove it in the isolated rabbit basilar artery, we compared the effects of sildenafil with other PDE-5 inhibitors, assessed the endothelial dependence of the vasoactive responses, and used modulators of the cGMP and cAMP signaling processes. Sildenafil (10 nM–0.1 mM) induced concentration-dependent relaxations of endothelin-1 (10 nM)-precontracted basilar artery, which were partially inhibited both in endothelium-denuded arteries and in arteries precontracted by depolarization with KCl (50 mM). Endothelin-1 (1 pM–30 nM) induced concentration-dependent contractions that were inhibited by sildenafil (0.1–100 μM). Zaprinast (10 nM–0.1 mM) and MBCQ (1 nM–0.1 mM), PDE-5 inhibitors, induced concentration-dependent relaxations with lower and higher potency than sildenafil, respectively. Sildenafil-induced relaxation was inhibited in arteries preincubated with the NO synthase inhibitor L-NAME (0.1 mM) or the soluble guanylyl cyclase inhibitor ODQ (10 μM). Preincubation with sildenafil (0.1 μM) enhanced the relaxations induced by acetylcholine (0.1 nM–0.1 mM) and the NO donor sodium nitroprusside (0.1 nM–0.1 mM), but not those induced by the cell-permeable cGMP analogue 8-Br-cGMP (1 nM–0.1 mM) and the adenylyl cyclase activator forskolin (0.1 nM–10 μM). These results show that sildenafil has vasoactive effects in isolated cerebral arteries. By enhancing the NO-cGMP signaling pathway in the cerebrovascular wall, sildenafil induces vasodilation, prevents vasoconstriction, and potentiates the effect of other NO-dependent vasodilators.

Keywords: Cerebral arteries; cGMP; PDE-5; PDE inhibitors; Sildenafil; Vasodilation

1. Introduction

The treatment of erectile dysfunction has been radically changed by selective inhibitors of phosphodiesterase-5 (PDE-5). Since its introduction in 1998, sildenafil (Viagra®) has been used by more than 20 million men in over one hundred countries. Clinically demonstrated efficacy and safety of this convenient oral treatment have displaced unpleasant intracavernosal or intrauretral injections of vasoactive substances (Gresser and Gleiter, 2002).

The enhancement of penile erection by sildenafil involves selective PDE-5 inhibition, cGMP breakdown reduction, and subsequent potentiation of the nitric oxide (NO)-stimulated cGMP signal mediating smooth muscle relaxation of both corpus cavernosum (Ballard et al., 1998) and penile vessels (Medina et al., 2000b). However, since penile smooth muscle is not the sole tissue expressing PDE-5, typical side effects of sildenafil are headache and facial flushing due to vasodilation, and dyspepsia due to relaxation of gastroesophageal sphincter (Lim et al., 2002). On the other hand, since selectivity of sildenafil for PDE-5 is not absolute, occasional transient visual disturbances have been described as a result of PDE-6 inhibition in retinal rods after relatively high dosages (Carson, 2003).

Relevant to cerebral blood flow (CBF) and function in health and disease, vasoactive and other potentially beneficial effects of more or less selective PDE inhibition are not new. In organ-bath type studies, selective inhibitors of PDE-1 (Kruuse et al., 2001), PDE-3 (Birk et al., 2004a; Shiraishi et al., 1998), PDE-4 (Birk et al., 2004a; Willette et al., 1997), and PDE-5 (Kruuse et al., 2001) induce relaxation of isolated cerebral arteries. In vivo studies have shown that topical application of

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non-selective and selective PDE inhibitors induce increases in cerebrospinal fluid levels of both cAMP and cGMP, and dilation of pial arterioles (Parfenova et al., 1993; Rosenblum et al., 1993). No change in CBF has been reported after non-PDE selective or PDE-5-selective inhibition (Kruuse et al., 2000; Wei et al., 1996), while selective PDE-3 inhibition with olprinone (Yu et al., 2000) and cilostazol (Birk et al., 2004b) have reached conflicting results. With regard to stroke, PDE inhibition in animal models of cerebral ischemia reduces thrombi formation and improves microcirculation (Tanaka et al., 1989), protects against blood–brain barrier disruption (Belayev et al., 1998), increases CBF (Turcani and Tureani, 2001) and reduces infarct size (Johnson et al., 1998). Finally, the selective PDE-5 inhibitor zaprinast restores NO-dependent cerebral vasodilator responses impaired after subarachnoid hemorrhage (SAH) (Sobey and Quan, 1999). In the clinical arena, the non-specific PDE inhibitor papaverine (Kaku et al., 1992), and more recently the PDE-3 inhibitor milrinone (Arakawa et al., 2001), have been used to reverse SAH-induced vasospasm.

If we focus on sildenafil, transient increases in CBF have been described in rats after oral administration (Zhang et al., 2002). However, intake of sildenafil doses used to treat erectile dysfunction failed to induce changes in either middle cerebral artery diameter, blood velocity, or CBF in both men and women (Kruuse et al., 2002, 2003; Arnavaz et al., 2003). Therapeutical applications in cerebrovascular disorders are being suggested as sildenafil induces neurogenesis and promotes functional recovery after focal ischemia in rats (Zhang et al., 2002), and reverses vasospasm after SAH in dogs (Inoha et al., 2002). However, the vasoactive effects of sildenafil in isolated cerebral arteries have not been studied to date.

The aim of this study was to assess the possible vasoactive effects of sildenafil in the isolated rabbit basilar artery. We hypothesized that sildenafil, as a selective PDE-5 inhibitor, interacts with the NO-cGMP pathway in the cerebral arteries and induces or potentiates vasodilation. To prove it, we compared the effects of sildenafil with other selective PDE-5 inhibitors, assessed the endothelial dependence of the vasoactive response, and used selective modulators of the cGMP and cAMP signaling processes.

2. Materials and methods

Experiments were conducted in compliance with the Spanish legislation on “Protection of Animals used for Experimental and other Scientific Purposes”, and in accordance with the Directives of the European Community on this subject.

2.1. Tissue preparation

Sixty two male New Zealand White rabbits (Technology Transferring Center, Polytechnic University of Valencia, Spain), weighing 2.5–3 kg, were killed by injection of 25 mg kg^{-1} sodium thiopental (Tiobarbital, B Braun Medical, Jaén, Spain) and 1.5 ml of 10 mM KCl solution through the ear vein. The whole brain, including the brainstem, was removed. The basilar artery was dissected free and cut in four 3-mm long segments, some of which were mechanically devoid of endothelium by gentle rubbing with a stainless-steel rod introduced through the arterial lumen. For isometric tension recording, the segments were mounted in an organ bath by using tungsten wires 89 μm in diameter. Two pins were introduced through the arterial lumen: one pin was fixed to a stationary support, while the other one was connected to a strain gauge (Universal Transducing Cell UC3, Gould Statham, Oxnard, CA, USA). Isometric tension was conveniently amplified (OCTAL Bridge, ADInstruments, Castle Hill, Australia), digitized (PowerLab/8SP, ADInstruments), recorded and stored in an IBM® PC compatible computer by means of the appropriate software (Chart 5, ADInstruments) for later analysis. Each organ bath contained 5 ml of Ringer–Locke solution at 37 °C and bubbled with a 95% O_2 and 5% CO_2 mixture to give a pH of 7.3–7.4. Previously determined optimal resting tension of 0.5 g was applied to the arterial segments, and they were allowed to equilibrate for 30–60 min before starting the experiments.

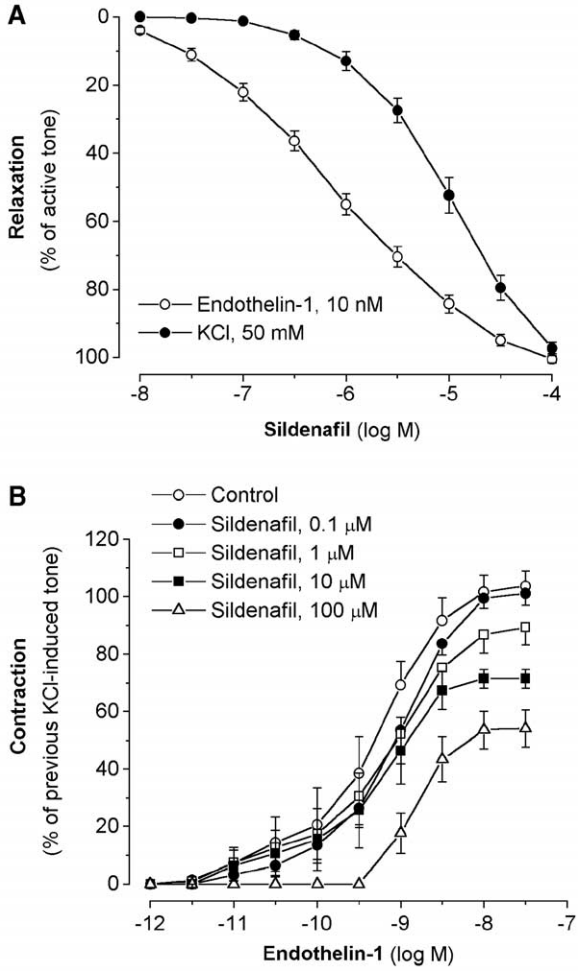

Fig. 1. (A) Concentration-dependent relaxations of endothelin-1-precontracted and KCl-precontracted rabbit basilar artery to sildenafil; (B) concentration-dependent contractions of rabbit basilar artery to endothelin-1, in control conditions and during incubation with increasing concentrations of sildenafil. Data are means ± S.E.M.