Sildenafil acts on the central nervous system increasing sympathetic activity

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Departments of 1Physiology and 2Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, and 3Department of Biological Sciences, Federal University of the Triângulo Mineiro, Uberaba, Minas Gerais, Brazil

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Fazan R Jr, Huber DA, Silva CA, Dias da Silva VJ, Salgado MC, Salgado HC. Sildenafil acts on the central nervous system increasing sympathetic activity. J Appl Physiol 104: 1683–1689, 2008. First published April 3, 2008; doi:10.1152/japplphysiol.01142.2007.—Sildenafil induces vasodilation and is used for treating erectile dysfunction. Although its influence on resting heart function appears to be minimal, recent studies suggest that sildenafil can increase sympathetic activity. We therefore tested whether sildenafil injected into the central nervous system alters the autonomic control of the cardiovascular system in conscious rats. The effect of sildenafil citrate injected into the lateral cerebral ventricle was evaluated in conscious rats by means of the recording of lumbar sympathetic nerve activity (LSNA), spectral analysis of systolic arterial pressure and heart rate variability, spontaneous baroreflex sensitivity, and baroreflex control of LSNA. Intracerebroventricular (ICV, 100 µg/5 µl) administration of sildenafil caused remarkable tachycardia without significant change in basal arterial pressure and was associated with a conspicuous increase (47±14%) in LSNA. Spectral analysis demonstrated that systolic arterial pressure oscillations in the low frequency (LF) range were increased (from 6.3±1.5 to 12.8±3.8 mmHg2), whereas the high frequency (HF) range was not affected by ICV administration of sildenafil. Sildenafil increased pulse interval oscillations at LF and decreased them at HF. The LF-HF ratio increased from 0.04±0.01 to 0.17±0.06. Spontaneous baroreflex sensitivity measured by the sequence method and the baroreflex relationship between mean arterial pressure and LSNA were not affected by ICV administration of sildenafil. In conclusion, sildenafil elicited an increase in sympathetic nerve activity that is not baroreflex mediated, suggesting that this drug is able to elicit an autonomic imbalance of central origin. This finding may have implications for understanding the cardiovascular outcomes associated with the clinical use of this drug.

Sildenafil is an effective therapy for erectile dysfunction, a condition that affects more than 100 million men worldwide (15, 32, 51). Sildenafil is an orally active vasodilator that prolongs the effect of cGMP by selectively inhibiting cGMP-phosphodiesterase type 5 (PDE5), the predominant isoenzyme in the corpus cavernosum (6, 51). Sildenafil citrate is well tolerated and has a favorable side-effect profile, with most side effects being associated with vasodilation (34). After a single therapeutic dose, a dose-independent mild and transient hypotension is often observed (18). The effects of sildenafil administration on heart rate (HR) are either no effect or a small tachycardia, attributed to baroreflex activation (1, 18, 40). Nevertheless, temporally related cardiovascular outcomes after sildenafil use, including myocardial infarction, arrhythmias, and sudden death, raised serious concern regarding the safety of this drug (14b).

Despite the fact that no changes in sympathetic activity have been demonstrated after sildenafil administration (1, 12), there are reports that sildenafil induces an increase in muscle sympathetic nerve activity both at rest and during physical, mental, or metabolic stress in healthy volunteers (39), as well as an increase in plasma catecholamine levels (41). Thus we hypothesize that sildenafil may have a direct central effect on sympathetic drive. This potential mechanism is supported, in part, by the evidence that sildenafil crosses the blood-brain barrier and that PDE5 is present inside the brain (4, 29, 47).

To our knowledge, there have been no reports of the hemodynamic and autonomic effects of sildenafil injected into the central nervous system (CNS). Therefore, this study aims to evaluate the effects of sildenafil citrate, injected into the lateral cerebral ventricle of conscious rats, on arterial pressure, HR, and lumbar sympathetic nerve activity (LSNA). In addition, the central effects of sildenafil citrate on arterial pressure and HR variability, as well as on spontaneous baroreflex sensitivity, were also investigated with a noninvasive method to evaluate effects on autonomic control of the cardiovascular system.

MATERIALS AND METHODS

The experiments were conducted on 31 conscious male Wistar rats (200–220 g) divided into groups: the first group was used to investigate cardiovascular variability (17 animals); the second group was used to investigate LSNA (14 animals). The animals were housed individually with free access to food and water and were maintained on a 12:12-h light-dark cycle; at 22°C. During the experiments, silence was carefully maintained to avoid stressing the animals. At the end of the experiments, the animals were killed by means of an intravenous overdose of pentobarbital sodium. All experimental protocols used in this study were reviewed and approved by the Animal Care and Use Committee of the School of Medicine of Ribeirão Preto of the University of de São Paulo.

Placement of the guide cannula into the left lateral cerebral ventricle. Four days before the experiments, the animal was anesthetized with tribromoethanol (2.5 mg/kg ip) and placed in a stereotaxic apparatus (David Kopf, Tujunga, CA) for the implantation of an intracerebroventricular (ICV) guide cannula positioned into the left lateral cerebral ventricle. To implant the cannula, a small window was opened in the region of bregma through which a 10-mm-long 23-gauge stainless-steel guide cannula was implanted according to the following coordinates relative to bregma: anterioposterior: 1.5 mm, lateral: 1.6 mm, ventral: 2.7 mm, and inclination: −2.5 mm. The guide cannula was fixed to the skull with methacrylate, attached to

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LSN recording. On the day of the experiment, the animal was anesthetized with tribromoethanol (2.5 mg/kg ip), and the femoral artery and vein were catheterized with polyethylene tubing for arterial pressure recording and intravenous drug administration, respectively. Under the same anesthesia, the left LSN was exposed after laparotomy, following careful dissection; the nerve was then placed on a bipolar stainless steel pair of electrodes and secured with a polyvinylsloxane impression material (Super-Dent; Carlisle Laboratories, Rockville Centre, NY). The electrode endings were exteriorized along with the vascular catheters in the animal’s nape. All incisions were sutured, and the animal was allowed to recover. Six to eight hours after surgery, the experimental protocol was carried out with the animal moving freely inside the cage. Left LSN was properly amplified (500–1,000×), band pass filtered (10 Hz to 3 kHz) using a differential High-impedence Cyberamp 320 amplifier (Axon Instruments, Foster City, CA) and digitally recorded (10 kHz) in an IBM/PC equipped with an analog-to-digital interface (CAD 12/36; Lynx Eletronica, SP, Brazil). LSN was integrated every 10 ms, and background noise, determined after bolus injection of phenylephrine (4 μg/kg iv), was subtracted. To compare multifractal features from different rats, LSN was normalized as a function of basal activity (basal = 100%). After basal recording of arterial pressure and LSN, the rat received an ICV injection of sildenafil (100 μg/5 μl, n = 9) or vehicle (n = 8), and simultaneous recording of arterial pressure and LSN continued for 60 min. Baroreflex control of LSN was determined in response to infusion of phenylephrine and sodium nitroprusside, before and after (60 min) ICV administration of sildenafil or vehicle. To determine the mean arterial pressure (MAP) vs. LSN curve, these parameters were recorded during the increase in arterial pressure, elicited by a bolus injection of phenylephrine (4 μg/kg iv) until complete disappearance of the action potentials of LSN, associated with bradycardia. Next, after LSN returned to the basal level, following arterial pressure normalization, recording of LSN continued during the decrease in arterial pressure elicited by a bolus injection of sodium nitroprusside (32 μg/kg iv), reaching maximal activity of LSN, associated with tachycardia. Thus the MAP vs. LSN curve was plotted and fitted by four-parameter nonlinear sigmoidal regression. The maximum slope (gain) of the LSN curve was calculated from the first derivative of the sigmoidal fit. Two other important parameters describing the baroreflex control of LSN were the MAP corresponding to the maximum LSN (MAPmax) and the MAP corresponding to the disappearance of LSN (MAPmin). These parameters were obtained by the MAP corresponding to the first (MAPmax) and second (MAPmin) points of maximal inflexion of the sigmoidal fit of the MAP vs. LSN relationship, identified by its third derivative.

Statistical analysis. Results are presented as means ± SE. Baseline values of PI and SAP, as well as variance, and LF and HF power of PI and SAP, were compared between groups (sildenafil or vehicle) using the nonparametric Mann-Whitney test. Differences in all parameters before and after treatment inside the groups were assessed by the Wilcoxon test. Two-way ANOVA for repeated measurements, followed by Tukey’s test, was performed to compare the time course of MAP, HR, and LSN between two experimental groups. Differences were considered statistically significant at P < 0.05.

RESULTS

Hemodynamics. ICV injection of sildenafil did not affect SAP but significantly increased HR, whereas vehicle injection induced no detectable changes in these parameters (Table 1). A typical recording of the effect elicited by sildenafil on arterial pressure and HR is illustrated in Fig. 1.

SAP and HR variability. Spectral analysis revealed two major oscillatory components for either SAP or PI. Under basal recordings, the frequency of slower oscillations of these car-
diovascular parameters ranged between 0.38 and 0.56 Hz (LF), whereas faster oscillations ranged between 1.8 and 2.1 Hz (HF). Neither sildenafil nor vehicle changed the frequency of the oscillatory component determined in SAP or HR spectra (data not shown). Group data of variance and the power of oscillatory components of SAP and PI, before and after ICV administration of sildenafil or vehicle, are shown in Table 1. Sildenafil increased the power of LF oscillations of either SAP or PI. The power of the HF component of SAP was not affected by sildenafil, whereas the power of HF oscillations of PI was reduced. ICV administration of sildenafil elicited a significant increase in the LF-HF ratio of PI.

**Spontaneous baroreflex activity.** The number and the slope of spontaneous baroreflex sequences were not affected by ICV administration of sildenafil or vehicle (Fig. 2).

**LSNA.** ICV injection of sildenafil induced an increase in LSNA combined with no change in arterial pressure, as illustrated in Fig. 3. Vehicle administration did not change the variables examined. Group data of MAP, HR, and LSNA before and after ICV administration of sildenafil or vehicle are displayed in Fig. 4. Overall, these data show that ICV administration of sildenafil increased both LSNA and HR without affecting MAP, whereas vehicle administration did not affect any of the variables examined throughout the 60-min period of observation. Baroreflex sensitivity in the control of LSNA was not affected by ICV administration of sildenafil or vehicle, as shown in Fig. 5 and Table 2.

**DISCUSSION**

The major findings of the present study are that ICV administration of sildenafil caused a conspicuous increase in LSNA combined with tachycardia without any change in arterial pressure. In addition, data from spectral analysis provided support for an increased sympathetic drive by means of the clear-cut demonstration of an increased power of LF oscillations of either SAP or PI, combined with an increased LF/HF ratio of PI. Finally, since ICV administration of sildenafil did not affect arterial pressure or baroreflex sensitivity, the observed sympathetic activation elicited by sildenafil injected into the CNS was independent of the baroreflex.

**Fig. 1.** Typical tracings of pulsatile arterial pressure (PAP) and mean arterial pressure (MAP) (white line) and heart rate (HR) before (basal) and after intracerebroventricular (ICV) administration of vehicle (top) or sildenafil (bottom).

**Fig. 2.** Number of sequences per 1,000 heart beats (A) and baroreceptor-HR reflex gain (B) before and after ICV administration of vehicle (n = 8) or sildenafil (n = 6) in conscious rats. Data are means ± SE.
There are a number of reports that examined the effect of sildenafil on arterial pressure and HR in healthy volunteers (16, 18, 39, 41, 51). Modest nondose-related decreases in arterial pressure were observed in healthy volunteers after oral or intravenous administration of single doses of sildenafil; these were not accompanied by changes in HR (51). Nevertheless, it has been reported that oral administration of sildenafil elicits increases in muscle sympathetic nerve activity and plasma catecholamines without affecting arterial pressure and HR (39). Because sildenafil crosses the blood-brain barrier and is able to inhibit PDE5 in cerebral blood vessels (29, 47), the sympathetic activation after oral administration of sildenafil (39) could be due to a direct effect on the CNS. The present study gives support for this hypothesis since direct administration of sildenafil into the CNS of conscious rats caused tachycardia, as well as increase in the LF power of both SAP and PI spectra and increases in LSNA without change to the baroreflex.

Spontaneous variability of cardiovascular parameters, particularly SAP and HR, examined in time and frequency domains (spectral analysis) has emerged as a reliable noninvasive method for evaluation of autonomic control of the cardiovascular system (25, 36, 45). A significant increase in the LF power of SAP spectra was found in the present study after central administration of sildenafil. Arterial pressure oscillations in the LF range are widely used as an index of sympathetic modulation of vascular tone (7, 35). Data from SAP variability, such as those obtained in the present study, strongly suggest an autonomic imbalance toward sympathetic activation. Interpretation of HR variability spectra is more complex than the interpretation of arterial pressure variability spectra because HR variability takes into account the interplay of sympathetic and vagal influences on the heart. The decreased HF power in PI spectra, observed after ICV administration of sildenafil, most likely represents decreased cardiac parasympathetic activity. On the other hand, LF oscillations of PI certainly involve a greater involvement of the sympathetic drive (30, 35) although vagal modulation of the sinus node in the LF oscillations of PI should also be taken into account (17, 19).

The observed increases in HR and LSNA elicited by ICV administration of sildenafil in conscious rats did not result from activation of the baroreflex since no changes were observed in arterial pressure or spontaneous baroreflex sensitivity. The sequence method used in the present study has been described as a valid and highly reliable approach to estimating spontaneous baroreflex sensitivity (33, 43, 48). Moreover, injection of
sildenafil into the CNS also had no effect on the MAP vs. LSNA relationship. The increased HR and LSNA associated with unaffected arterial pressure and baroreflex sensitivity after central administration of sildenafil citrate may represent regional variability in gain, range, and the maximal inhibition exerted by baroreflex regulation of sympathetic activity (42). In addition, it has been demonstrated in conscious rats that air-jet stress caused modest increases in arterial pressure, tachycardia, renal and mesenteric vasoconstriction, and marked hindquarter vasodilation, mediated by active sympathetic neurogenic vasodilatation involving the release of nitrosyl factors from postganglionic LSN fibers and/or the vascular endothelium (14a). Therefore, it can be hypothesized that, in the present study, a combined increase in the sympathetic activity to the heart and presumably to other vascular territories, e.g., renal and mesenteric, were compensated by sympathetic neurogenic vasodilation in the hindquarters, resulting in no overall change in arterial pressure. PDE5 is likely to be important in determining intracellular cGMP levels, thus activating cGMP-dependent signaling pathways. There is a body of evidence that a cGMP/PDE5 pathway plays an important role in vascular relaxation but also acts on several functions within the CNS (20). Sildenafil elevates the brain levels of cGMP in normal rats, inducing neurogenesis (49, 50). Although it has been demonstrated that the highest levels of PDE5a mRNA are found in the cerebellum (20), the effect of sildenafil observed in the present study might be uncovering a role for a cGMP/PDE5 pathway in areas that control the sympathetic drive (21). However, an inhibitory effect on other PDE isoforms present in the brain, such as PDE1 and especially PDE10, cannot be excluded (22). Endogenous nitric oxide (NO) is an activator of the soluble cGMP-synthesizing enzyme guanylate cyclase, and there has been much controversy about the role of NO in controlling sympathetic nerve activity and cardiovascular function (21). For example, application of NO precursors, or donors, into the nucleus of the solitary tract of rats elicits inhibitory (37, 46) or excitatory (27) effects on sympathetic nerve activity. Likewise, administration of NO into the rostral ventrolateral medulla, a medullary site containing neurons that project to the sympathetic preganglionic neurons (SPN) of the spinal cord (11, 21) and is considered as the final common pathway for regulating sympathetic drive (10, 13, 28), also demonstrated that NO seems to elicits inhibitory (21, 38) or excitatory (8, 26, 31) effects on sympathetic nerve activity in rats. Another important site of action of NO in controlling arterial pressure and HR is the SPN (2, 5) from the thoracic spinal cord (24). Intrathecal administration of the membrane-permeant 8-bromo cGMP, or the selective inhibitor of the soluble form of guanylate cyclase ODQ, indicates that cGMP increases sympathetic nerve activity in anesthetized rats, providing support to the hypothesis that the sympatho-excitatory effects of spinal delivery of NO are mediated by a cGMP-dependent mechanism (24). The results obtained with ICV administration of sildenafil point to a role for cGMP in the CNS, producing an increase in sympathetic nerve activity. The disadvantage of this approach is that the injection of sildenafil was made into the cerebrospinal fluid, preventing the possibility of precisely localizing where this selective inhibitor of PDE5 was acting. It is well described that acute use of sildenafil for penile erectile dysfunction may cause arterial hypotension and reflex tachycardia due to autonomic imbalance (39), which may lead to cardiovascular risk due to severe cardiac arrhythmias and even sudden death (3, 9). Such side effects are more intense in patients with resting sympathetic overactivity, like those presenting congestive heart failure or under use of vasodilators such as organic nitrates, which potentiate the NO-cGMP pathway (3, 9). Our results showing that the tachycardic response observed after ICV administration of sildenafil, associated with increased sympathetic nerve activity, may have clinical implications for understanding the cardiovascular outcomes associated with the clinical use of sildenafil, especially in patients with comorbidities.

In conclusion, the present study demonstrated for the first time that sildenafil elicited an increased sympathetic drive imposed on the cardiovascular system and that this effect is not baroreflex mediated. The administration of sildenafil into the CNS strongly suggests that this drug is able to elicit an autonomic imbalance of central origin in conscious and freely moving rats, which may have implications for understanding the cardiovascular outcomes associated with the clinical use of this drug.

Table 2. Effect of sildenafil administration on baroreflex sensitivity

<table>
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<tr>
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<th>Vehicle (n = 8)</th>
<th>Sildenafil (n = 9)</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>60 min</td>
</tr>
<tr>
<td>Gain, % LSNA/mmHg</td>
<td>–11.6±1.4</td>
<td>–9.7±1.3</td>
</tr>
<tr>
<td>Range, % LSNA</td>
<td>442±67</td>
<td>446±58</td>
</tr>
<tr>
<td>MAPbaso, mmHg</td>
<td>94±6</td>
<td>90±4</td>
</tr>
<tr>
<td>MAPcso, mmHg</td>
<td>108±2</td>
<td>117±6</td>
</tr>
<tr>
<td>MAPcso, mmHg</td>
<td>71±6</td>
<td>63±5</td>
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Data are means ± SE. MAP, mean arterial pressure; LSNA, lumbar sympathetic nerve activity; n, number of rats.
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REFERENCES


