Analysis of Erectile Responses to BAY 41-8543 and Muscarinic Receptor Stimulation in the Rat

George F. Lasker, MS,* Edward A. Pankey, MD,* Alexander V. Allain, MS,* Jasdeep S. Dhaliwal, MD,* Johannes-Peter Stasch, PhD,† Subramanyam N. Murthy, PhD,* and Philip J. Kadowitz, PhD*

*Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA, USA; †Institute of Cardiovascular Research, Pharma Research Centre, Bayer AG, Wuppertal, Germany

DOI: 10.1111/j.1743-6109.2012.02912.x

ABSTRACT

Introduction. Soluble guanylate cyclase (sGC) is the receptor for nitric oxide (NO) and in pathophysiologic conditions where NO formation or bioavailability is impaired, erectile dysfunction (ED) occurs.
Aim. The aim of this study was to investigate erectile responses to the sGC stimulator BAY 41-8543 in physiologic and pathophysiologic conditions.
Methods. Increases in intracavernosal pressure (ICP) in response to intracavernosal (ic) injections of BAY 41-8543 were investigated in the anesthetized rat.
Main Outcome Measures. Increases in ICP/MAP in response to ic injections of BAY 41-8543 and the interaction of BAY 41-8543 with exogenous and endogenously released NO were investigated and the effect of the sGC stimulator on cavernosal nerve injury was assessed. The mechanism of the increase in ICP/MAP in response to ic injection of acetylcholine was investigated.
Results. The ic injections of BAY 41-8543 increased ICP/MAP and the duration of the response. BAY 41-8543 was less potent than sodium nitroprusside (SNP) and ic injections of BAY 41-8543 and SNP produced a larger response than the algebraic sum of responses to either agent alone. Simultaneous ic injection of BAY 41-8543 and cavernosal nerve stimulation produced a greater response than either intervention alone. Atropine and cavernosal nerve crush injury decreased the response to nerve stimulation and ic injection of BAY 41-8543 restored the response.
Conclusion. These data show that BAY 41-8543 has significant erectile activity and can synergize with exogenous and endogenously released NO. This study shows that atropine and nerve crush attenuate the response to cavernosal nerve stimulation and that BAY 41-8543 can restore the response. The results with atropine, L-NAME and hexamethonium indicate that the response to ic injection of acetylcholine is mediated by muscarinic receptors and the release of NO with no significant role for nicotinic receptors. These results suggest that BAY 41-8543 would be useful in the treatment of ED. **Lasker GF, Pankey EA, Allain AV, Dhaliwal JS, Stasch J-P, Murthy SN, and Kadowitz PJ. Analysis of erectile responses to BAY 41-8543 and muscarinic receptor stimulation in the rat. J Sex Med 2013;10:704–718.**

Key Words. sGC Stimulator; BAY 41-8543; Erectile Function; Muscarinic Receptors; Cavernosal Nerve Stimulation; Increased cGMP

Introduction

It is well established that nitric oxide (NO) is the principle mediator of penile erection. NO is released from the nerves innervating the penis and from the endothelium of the corpora cavernosa [1–3]. The release of NO from nerve terminals and the endothelium activates sGC, increases cGMP levels, and promotes penile erection [4–6]. ED is associated with diseases like hypertension, diabetes mellitus, atherosclerosis, and from pelvic nerve damage following prostatectomy [7–11]. These disease states are associated with decreased NO formation or bioavailability and PDE5 inhibitors have a beneficial effect but depend on an intact NO system [12–14]. A newer class of agents that

directly target sGC and increase cGMP formation independent of NO have been developed and would be useful in the treatment of ED when NO formation or bioavailability is impaired. These agents decrease platelet aggregation and promote vasodilation in isolated vessels and in the intact circulation [1,3]. It has been reported that the prototype sGC stimulator, YC-1, has erectile activity and enhances the erectile response to apomorphine and cavernosal nerve stimulation in the rat [2]. It has also been reported that a newer sGC stimulator, BAY 41-2272, has erectile activity in the rabbit and that responses were greatly enhanced with sequential administration of the NO donor sodium nitroprusside (SNP) [4]. BAY 41-8543 is a recently developed sGC stimulator reported to have greater selectivity and potency, and to exhibit synergy with NO over a wide range of concentration [5]. The purpose of the present study was to investigate erectile responses to BAY 41-8543 and to determine the interaction of BAY 41-8543 with endogenously released and exogenously administered NO. In addition the hypothesis that BAY 41-8543 would have a beneficial effect on erectile function after cavernosal nerve crush injury or muscarinic blockade was tested.

Materials and Methods

The Institutional Animal Care and Use Committee of Tulane University School of Medicine approved the experimental protocol used in these studies, and all procedures were conducted in accordance with institutional guidelines. For these experiments, adult male Sprague–Dawley rats, weighing 339–463 g, were anesthetized with Inactin (thiobutabarbital), 100 mg/kg i.p. Supplemental doses of Inactin were given i.p. as needed to maintain a uniform level of anesthesia. Body temperature was maintained with a heating lamp. The trachea was cannulated with a short segment of PE-240 tubing to maintain a patent airway, and the left carotid artery was catheterized with PE-50 tubing for measurement of systemic arterial pressure. Intracavernosal pressure (ICP) was measured with a 25-gauge needle inserted into the left crura of the penis connected to PE-50 tubing filled with heparin. Systemic arterial pressure and ICP were measured with Namic Perceptor DT pressure transducers and a data acquisition system (Biopac MP 100A-CE, Santa Barbara, CA, USA). Intracavernosal pressure, systemic arterial pressure, and mean systemic arterial pressure (MAP) obtained by electronic averaging were continuously recorded and were displayed and stored on a Dell PC [8,9]. The left jugular vein was catheterized with PE-50 tubing for the systemic administration of drugs and fluids. A 30-gauge needle connected to PE-10 tubing was placed in the right crura of the penis for administration of BAY 41-8543, SNP, and ACh. Maximal ICP in response to ic injection of the vasodilator agents or in response to cavernosal nerve stimulation was measured at the peak of the erectile response. The area under the curve (AUC) and duration of the increase in ICP were measured to characterize the total erectile response.

Cardiac output was measured by the thermodilution technique with a Cardiomax II computer (Columbus Instruments, Columbus, OH, USA). A known volume (0.2 mL) of room temperature 0.9% NaCl solution was injected into the jugular vein catheter with the tip near the right atrium and changes in blood temperature were detected by a 1.5F thermistor microprobe catheter (Columbus Instruments) positioned in the aortic arch from the left carotid artery. Heart rate was measured by the Biopac from the pressure pulse interval data.

Cavernosal nerve stimulation was performed as previously described in the literature [10]. For nerve stimulation, the bladder and prostate were exposed through a midline abdominal incision. The cavernosal nerve was identified posterolateral to the prostate on one side, and a stainless steel bipolar stimulating electrode was placed on the nerve. The cavernosal nerve was stimulated with square wave pulses at frequencies of 2, 4, 8, 10, and 16 Hz, at 5V and a pulse width of 5 ms for a duration of 60 seconds with a Grass Instruments SD9 Stimulator (Quincy, MA, USA). A rest period of at least 3 minutes was allowed between nerve stimulation trials and similar results were obtained with 5- and 10-minute rest periods. Nerve crush experiments were performed with three 15-second applications of a forcep to the cavernosal nerve.

Vagal stimulation was performed by dissecting the right vagus nerve away from the adjacent carotid artery. A bipolar stimulating electrode was placed around the nerve and stimulated with square wave pulses at a frequency of 16 Hz at 5V and a pulse width of 5 ms for a duration of 15 seconds with a Grass Instruments SD9 Stimulator. The vagus was ligated rostral of the electrode with a 4-0 silk suture to prevent retrograde stimulation. A rest period of 10 minutes was allowed between nerve stimulation trials.

The experiments in this study were designed to: (i) characterize increases in ICP/MAP in response