Published in final edited form as:

J Sex Med. 2013 March ; 10(3): 704–718. doi:10.1111/j.1743-6109.2012.02912.x.

ANALYSIS OF ERECTILE RESPONSES TO BAY 41-8543 AND MUSCARINIC RECEPTOR STIMULATION IN THE RAT

George F. Lasker, M.S.[£], Edward A. Pankey, M.D.[£], Alexander V. Allain, M.S.[£], Jasdeep S. Dhaliwal, M.D.[£], Johannes-Peter Stasch, Ph.D.[¶], Subramanyam N. Murthy, Ph.D.[£], and Philip J. Kadowitz, Ph.D.[£]

[£]Department of Pharmacology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, Louisiana 70112-2699 USA

[¶]Institute of Cardiovascular Research, Pharma Research Centre, Bayer AG, Wuppertal, Germany

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 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.

Corresponding Author: Philip J. Kadowitz, Ph.D., Department of Pharmacology, SL83, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, Louisiana 70112-2699 USA, Phone: 504 988-2634, Fax: 504 988-5283, pkadowi@tulane.edu.

Conflict of Interest: None

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 cavernosa¹⁻³. The release of NO from nerve terminals and the endothelium activates sGC, increases cGMP levels and promotes penile erection⁴⁻⁶. ED is associated with diseases like hypertension, diabetes mellitus, atherosclerosis, and from pelvic nerve damage following prostatectomy⁷⁻¹¹. These disease states are associated with decreased NO formation or bioavailability and PDE-5 inhibitors have a beneficial effect but depend on an intact NO system¹²⁻¹⁴. 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 rat². 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 concentration⁵. 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 339–463g, 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, 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). 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 literature¹⁰. 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. 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 to ic injection of a wide range of doses of the sGC stimulator, BAY 41-8543, (ii) to compare responses to ic injections of BAY 41-8543 with responses to sodium nitroprusside (SNP), (iii) to investigate the role of muscarinic receptor activation in mediating increases in ICP in response to cavernosal nerve stimulation and investigate the mechanism by which ic injection of ACh increases ICP, (iv) to investigate the interaction of BAY 41-8543 with endogenously released and exogenously administered NO, and (v) to determine if BAY 41-8543 had a beneficial effect on the response to cavernosal nerve stimulation following nerve crush injury.

In the first set of experiments erectile responses to ic injections of BAY 41-8543 (0.1–100 µg/kg) and sodium nitroprusside (SNP) (0.01-10 µg/kg) were compared and changes in ICP, MAP, the ratio of ICP/MAP at maximum ICP, the duration of the increase in ICP and area under the ICP curve (AUC) were measured when doses of the agents were expressed on a nmol/kg basis to take differences in molecular weight into account. Intracavernosal (ic) injections of the vehicle for BAY 41-8543 and SNP into the right crura had no significant effect on ICP.

In the second set of experiments, the effect of the muscarinic receptor antagonist atropine on the response to cavernosal nerve stimulation and exogenously administered ACh were investigated. The nerve was stimulated before and 10 minutes after administration of atropine, 1 mg/kg iv, and then an ic injection of BAY 41-8543, 0.1 µg/kg, was administered in the atropine treated animal to determine if the sGC stimulator could restore the erectile response to cavernosal nerve stimulation after muscarinic receptor blockade. The extent of muscarinic receptor blockade and the specificity of the blockade were assessed by iv and ic injections of ACh and SNP before and after atropine administration.

In the third set of experiments, the effects of the NOS inhibitor L-NAME and the nicotinic receptor antagonist hexamethonium on the response to ic injection of ACh was investigated to provide information on ACh mechanism of action in the corpora cavernosa.

In the fourth set of experiments the interaction between exogenously released NO and BAY 41-8543 was investigated. In these experiments ic injection of a low dose of the NO donor SNP (0.01 µg/kg) was given alone and together with BAY 41-8543 (0.3 µg/kg) and

responses to ic injections of SNP and BAY 41-8543 when given alone and in combination were compared.

In the fifth set of experiments, the interaction between endogenously released NO and BAY 41-8543 was investigated. In these experiments the effect of ic injection of BAY 41-8543 (0.1 $\mu\text{g/kg}$) during stimulation of the cavernosal nerve at 2 Hz was investigated.

In the last set of experiments the effects of BAY 41-8543 on the response to cavernosal nerve stimulation was investigated after nerve crush injury to determine if BAY 41-8543 had a beneficial effect in this pathologic model of ED.

BAY 41-8543 {2-[1-(2-fluorophenylmethyl)-1H-pyrazolo[3,4-b]pyridin-3-yl]-5(4-morpholinyl)-4,6-pyrimidinediamine} obtained from Dr. Johannes-Peter Stasch of the Institute of Cardiovascular Research, Pharma Research Centre, Bayer, Wuppertal, Germany, was dissolved in Transcutol-Cremophor EL-0.9% NaCl solution (10:10:80). No financial support was provided by Bayer. Sodium Nitroprusside, Acetylcholine Chloride, L-NAME, Hexamethonium Bromide and Atropine Sulfate (Sigma-Aldrich) were dissolved in 0.9% NaCl and solutions were made on a frequent basis. For ic injections the doses of BAY 41-8543, SNP and ACh were prepared in a total volume of 200 μl and were injected through the 30 gauge needle in the right crura. The data are expressed as mean $\pm$ SE and were analyzed using a one-way ANOVA and a student's t-test for paired data. A *p* value of less than 0.05 was used as the criterion for statistical significance.

Results

Effect of ic injections on erectile activity

The effect of ic injections of BAY 41-8543 on ICP/MAP, MAP, response duration and AUC was investigated in the anesthetized rat and these data are summarized in figure 1 and table 1. The ic injection of BAY 41-8543 in doses of 0.1–100 $\mu\text{g/kg}$ produced dose-related increases in ICP from 6 ± 1 to 48 ± 2 mmHg. A record from an experiment showing the effect of ic injection of BAY 41-8543, 100 $\mu\text{g/kg}$ on ICP, MAP, systolic and diastolic BP is shown in figure 1A. The ic injection of 200 μl of the vehicle for BAY 41-8543 had no significant effect on ICP. The changes in MAP in response to ic injections of BAY 41-8543 were measured at the time of the peak increase in ICP and again when the maximum fall in MAP occurred and these data are summarized in table 1. The maximum fall in MAP occurred after the peak increase in ICP and ranged from 2 ± 1 to 50 ± 3 mmHg and occurred in 56 ± 11 to 223 ± 36 seconds after ic injection (Table 1). The ICP to MAP ratio at maximum ICP increased in a dose-dependent manner (Fig. 1B) in response to ic injections of BAY 41-8543. MAP was decreased 1 ± 0 to 22 ± 4 mmHg at the time of the peak increase in ICP (Table 1). The increases in ICP in response to ic injections of BAY 41-8543 were rapid in onset (10–40 s) and the duration of the response was between 53 ± 8 and 2233 ± 268 seconds for doses of 0.1 and 100 $\mu\text{g/kg}$ (Fig. 1C). The total ICP response was determined by measuring the area under the ICP curve (AUC). The AUC increased in a dose-dependent manner in response to ic injections of 0.1 to 100 $\mu\text{g/kg}$ of BAY 41-8543 (Fig 1D). These results indicate that the sGC stimulator BAY 41-8543 has significant dose dependent erectile activity in the anesthetized rat.

Comparative effects of SNP and BAY 41-8543

Responses to ic injections of BAY 41-8543 and the NO donor sodium nitroprusside (SNP) were compared and these data are shown in figure 2A. The increases in ICP in response to BAY 41-8543 and SNP were compared with doses of the vasodilator agents expressed on a nmol/kg basis to account for differences in molecular weight. These results indicate that responses to SNP are significantly greater than responses to BAY 41-8543 at the higher

doses tested (Fig. 2A). The decreases in MAP in response to ic injections of BAY 41-8543 and SNP were also compared on a nmol/kg basis and show that decreases in MAP after ic administration of SNP were significantly greater than responses to BAY-41-8543 (Fig. 2B). The duration of the response to ic injections of BAY 41-8543 was longer when compared with the NO donor SNP (Fig. 2C). BAY 41-8543 elicited a significantly greater total erectile response (AUC) when compared to SNP (Fig. 2D). These results indicate that SNP is more potent on a molar basis than BAY 41-8543 in increasing ICP and decreasing MAP, however, responses to BAY 41-8543 were longer in duration when compared with SNP (Fig. 2).

Effect of muscarinic blockade on the response to cavernosal nerve stimulation

The role of muscarinic receptor activation in mediating the response to cavernosal nerve stimulation was investigated and stimulation of the nerves innervating the corpora at 2, 4, 8, 10 and 16 Hz produced stimulus-frequency related increases in ICP with the peak increase occurring at 10 Hz (Fig. 3A). Intracavernosal pressure increased 17 ± 5 , 49 ± 8 , 53 ± 9 , 62 ± 6 and 59 ± 6 mmHg at stimulus frequencies of 2, 4, 8, 10 and 16 Hz, respectively (Fig. 3A). The duration of the nerve stimulation trial was 1 minute at which time the increase in ICP reached a steady value with a rest period of at least 3 minutes between subsequent stimulations and similar data was obtained with 5 or 10 minute rest periods. The increase in ICP/MAP and AUC in response to cavernosal nerve stimulation at stimulation frequencies of 4, 8, 10 and 16 Hz was significantly attenuated after administration of atropine in a dose of 1 mg/kg iv (Fig. 3). The increase in ICP in response to ic injection of acetylcholine (ACh) was reduced significantly after treatment with atropine, 1 mg/kg iv, whereas the increase in ICP in response to ic injection of SNP was not changed (Fig. 4A and 4B). The decrease in MAP in response to iv injection of ACh was significantly attenuated after the administration of atropine 1 mg/kg iv while the decrease in MAP in response to iv injection of SNP was not altered (Fig. 4C and 4D). The decrease in MAP in response to iv injection of ACh occurred in the absence of a significant change in cardiac output or heart rate when values were measured at the peak of the fall in MAP (Fig 5). The data with ACh and atropine indicate that the increase in ICP and decrease in MAP are mediated by the activation of muscarinic receptors in the corpora cavernosa and in the systemic vascular bed. The absence of an effect of atropine on the change in ICP or MAP in response to SNP along with the ACh results indicate that the muscarinic receptor blockade is effective and specific in the penile and systemic vascular beds.

The role of nicotinic receptors in mediating increases in ICP in response to ic injections of ACh was investigated. The increase in ICP and the decrease in MAP in response to ic and iv injection of ACh was not significantly changed after administration of the nicotinic receptor blocking agent hexamethonium in a dose 2.5 mg/kg iv (Fig 6A). The extent of nicotinic receptor blockade by hexamethonium was assessed by investigating the inhibitory effect of the nicotinic receptor blocking agent on the response to efferent (preganglionic) vagal stimulation. Vagal stimulation decreased heart rate 125 ± 31 BPM before hexamethonium and 19 ± 3 BPM after hexamethonium indicating significant nicotinic receptor blockade ($p < 0.05$).

In order to provide information on the mechanism by which ACh increases ICP the effect of nonselective NOS inhibitor L-NAME on the response to ic injection of ACh was investigated. Following administration of L-NAME in a dose of 50 mg/kg iv the increase in ICP in response to ic injection of ACh was significantly attenuated (Fig 6B). The data with atropine, hexamethonium and L-NAME suggest that the response to ic injection of ACh is mediated by the activation of muscarinic receptors and the release of NO from the endothelium of the corpora cavernosum and that nicotinic receptor activation does not play an important role.

In order to determine if BAY 41-8543 can restore the response to cavernosal nerve stimulation after muscarinic receptor blockade, the effect of ic BAY 41-8543 injection and nerve stimulation were investigated. The ic injection of BAY 41-8543 (0.1 $\mu\text{g/kg}$) restored the increase in ICP in response to cavernosal nerve stimulation at 4 Hz to control value in animals treated with atropine (Fig. 7). The increase in ic pressure in response to nerve stimulation at 4 Hz was 48 ± 7 mmHg in the control period and after atropine treatment was 24 ± 4 mmHg. The erectile response was restored to 42 ± 4 mmHg by ic injection of BAY 41-8543 (Fig. 7). These data indicate that BAY 41-8543 has the ability to rescue the erectile response after muscarinic receptor blockade in the rat.

Synergy Experiments

In order to investigate the interaction between BAY 41-8543 and exogenously administered NO the erectile response to ic injection of a low dose of SNP (0.01 $\mu\text{g/kg}$) and BAY 41-8543 (0.3 $\mu\text{g/kg}$) were investigated. In these experiments SNP and BAY 41-8543 were injected separately and together in a single injection. The ic injection of SNP (0.01 $\mu\text{g/kg}$) and BAY 41-8543 (0.3 $\mu\text{g/kg}$) in separate injections produced increases in ICP of 6 ± 2 and 9 ± 1 mmHg respectively. The increase in ICP (24 ± 1 mmHg) and AUC in response to the combination injection of BAY 41-8543 and SNP were significantly greater than the algebraic sum of the individual responses to BAY 41-8543 and SNP (14 ± 2 mmHg) (Fig. 8A). All ic injections were made in a total fixed volume of 200 μl .

To investigate the interaction between BAY 41-8543 and endogenously released NO, the cavernosal nerve was stimulated at 2 Hz and then nerve stimulation was repeated with simultaneous ic injection of BAY 41-8543 (0.1 $\mu\text{g/kg}$). The ic injection of BAY 41-8543 during cavernosal nerve stimulation produced a significantly larger increase in ICP (38 ± 9 mmHg) and AUC than the algebraic sum of the response to the separate interventions (23 ± 4) (Fig. 8B). The results of these experiments indicate a synergistic interaction between BAY 41-8543 and exogenously administered NO and between BAY 41-8543 and endogenously released NO.

Effect of BAY 41-8543 after cavernosal nerve injury

The effect of ic injection of BAY 41-8543 on the increase in ICP in response to cavernosal nerve stimulation after nerve crush injury was investigated and these data are summarized in figure 9. Cavernosal nerve injury induced by forcep crush reduced the response to cavernosal nerve stimulation at 10 Hz by 83 %. A record from an experiment showing the effect of nerve crush injury and of ic injections of BAY 41-8543 on the attenuated response to cavernosal nerve stimulation is shown in figure 9A. The response to cavernosal nerve stimulation at 10 Hz did not recover spontaneously over the 4 hour period experiments were carried out ($n = 3$) (Fig 9B). The response to cavernosal nerve stimulation was restored to control value by ic injection of BAY 41-8543 1 $\mu\text{g/kg}$ during cavernosal nerve stimulation (Fig 9A, 9C, 9D and 9E). The results with atropine, L-NAME and cavernosal nerve crush injury can be interpreted to suggest that BAY 41-8543 may have a beneficial effect in the treatment of ED caused by diverse mechanisms.

Discussion

New findings in the present study are that BAY 41-8543 has significant erectile activity in the anesthetized rat. Although BAY 41-8543 was less potent than SNP on a nmol/kg basis in increasing ICP, erectile responses to the sGC stimulator were longer in duration and had a greater AUC than responses to the NO donor. Erectile responses to BAY 41-8543 were synergistic with endogenously released and exogenously administered NO suggesting that this agent would be useful in the treatment of ED resulting from decreased NO formation or

bioavailability. The response to cavernosal nerve stimulation was significantly reduced by atropine, and the response was restored by the ic administration of BAY 41-8543. Cavernosal nerve crush injury reduced the increase in ICP in response to cavernosal nerve stimulation by 83% and the response to cavernosal nerve stimulation was restored by ic injection of BAY 41-8543 suggesting that BAY 41-8543 may be useful in the treatment of ED resulting from nerve injury.

Results of the present study show that ic injections of the sGC stimulator BAY 41-8543 produce dose-related increases in ICP when injected into the corpora cavernosa in doses of 0.1–100 µg/kg. Increases in ICP were rapid in onset (10–40 sec) and long in duration (37 min) at the highest dose of the sGC stimulator studied. In terms of relative potency, when erectile responses are compared on a nmol/kg basis, BAY 41-8543 was less potent than SNP, and responses to the sGC stimulator were slower in onset and longer in duration than were responses to the NO donor.

The ic injection of BAY 41-8543 produced only small decreases in MAP at the time when the maximum increase in ICP occurred. However MAP decreased in a delayed fashion when the agent was absorbed into the systemic circulation after ic injection. The maximum decrease in MAP after the ic injection of BAY 41-8543 at the highest dose studied was greater by 28 mmHg when compared to the decrease in MAP at the point of maximum ICP and occurred 133 seconds after the measured increase in ICP (Table 1). The systemic absorption of vasoactive agents injected ic is delayed and significant decreases in MAP can be observed when most vasodilator agents are injected ic in large doses.

BAY 41-8543 is a heme dependent, NO independent, stimulator of sGC. In the present study, the interaction between BAY 41-8543 and NO was investigated in the corpora cavernosa of the anesthetized rat. The simultaneous injection of BAY 41-8543 and the NO donor SNP produced a significantly larger increase in ICP and in AUC than the algebraic sum of the responses to these agents when injected ic individually. These data indicate that BAY 41-8543 can synergize with exogenously administered NO in the corpora cavernosa of the anesthetized rat and suggest that low doses of BAY 41-8543 and SNP can be used to enhance erectile activity. The interaction of BAY 41-8543 and endogenously released NO was also investigated and the increase in ICP and AUC in response to cavernosal nerve stimulation and ic injection of BAY 41-8543 was significantly greater than the algebraic sum of the erectile responses when the two erectile stimuli were administered separately. These data indicate that BAY 41-8543 also synergizes with endogenously released NO suggesting that BAY 41-8543 could be used to enhance erectile responses that are impaired in pathologic conditions such as diabetes mellitus, hypertension or atherosclerosis where NO release or bioavailability is reduced^{7, 11, 15, 16}. The effect of BAY 41-8543 on erectile function was investigated when the increase in ICP in response to cavernosal nerve stimulation was impaired by nerve crush injury. Nerve crush induced by forcep pressure reduced the response to nerve stimulation by 83 %. The response to nerve stimulation did not spontaneously recover over the 4 hour period experiments were carried out. However the ic injection of BAY 41-8543 restored the response to cavernosal nerve stimulation to control value. These data are consistent with the hypothesis that BAY 41-8543 would be useful in the treatment of ED caused by nerve injury caused by surgical procedures. The data with SNP suggest that ic injection of BAY 41-8543 could be used along with an NO donor to enhance erectile function in patients with ED in situations where endogenous NO formation or bioavailability is reduced. The results with BAY 41-8543 are consistent with studies using YC-1, the prototypical sGC stimulator. The ic injection of YC-1 increased ICP and i.p. injections enhanced the response to cavernosal nerve stimulation in the rat². It has also been reported that YC-1 synergizes with NO in cavernosal tissue and potentiates the actions of the NO/cGMP pathway in the corpora cavernosa of the rat^{17, 18}. The results with BAY

41-8543 extend the results of previous studies with sGC stimulating agents by showing that ic injection of BAY 41-8543 can restore the response to cavernosal nerve stimulation after muscarinic receptor blockade and cavernosal nerve crush injury. Our results with BAY 41-8543 are consistent with the hypothesis that agents in this class will be useful in the treatment of ED caused by diverse mechanisms and are consistent with the concept that agents with improved selectivity and pharmacokinetics may have a therapeutic advantage when used in the treatment of ED. Studies in the rat show that BAY 41-8543 is well tolerated when administered on a chronic basis with the animals exhibiting normal grooming and feeding behavior. It has also been shown that BAY 41-8543 has an antiproliferative effect on smooth muscle cells in the urethra, bladder and prostate¹⁹.

NO is the principle mediator of penile erection and during the erectile response NO formed by nNOS is released from the nerves innervating the corpora cavernosum where ACh and nNOS may be colocalized²⁰. NO formed by eNOS is also released from the endothelium of the corpora cavernosum by the activation of muscarinic receptors by ACh released from cholinergic nerve terminals and mediates approximately 35% of the erectile response in the present study. The hypothesis that release of NO from nerves has an important role in the erectile response is based on studies showing that inhibitors of nNOS have a major inhibitory effect on the response to cavernosal nerve stimulation^{21, 22}. Although the role of NO released from nerves innervating the corpora cavernosa in mediating penile erection is well established, the role of muscarinic receptor activation by cholinergic nerve mediated release of NO is less well established²³. The contribution of muscarinic receptor stimulation in mediating the response to cavernosal nerve stimulation in the anesthetized rat was examined by investigating the effect of atropine. The increases in ICP, ICP/MAP and AUC in response to cavernosal nerve stimulation was reduced by an average of 40, 28 and 41% respectively at stimulus frequencies of 2, 4, 8, 10 and 16 Hz after treatment with the muscarinic receptor antagonist in a dose of 1 mg/kg iv. In these experiments, the increase in ICP in response to ic injection of ACh was significantly reduced whereas the increase in ICP in response to ic injection of SNP was not changed. The results with ic injections of ACh and SNP establish the efficacy and selectivity of the muscarinic receptor blockade produced by atropine. The present data suggests that the response to cavernosal nerve stimulation is mediated by the release of NO and ACh from cavernosal nerves. In addition, the fall in MAP in response to iv injections of SNP were not reduced after administration of atropine. The decrease in MAP in response to iv injection of ACh was associated with no significant change in cardiac output or heart rate when values were measured at the time of the peak decrease in MAP. These data indicate that the decrease in MAP in response to iv injection of ACh was caused by a cholinergically mediated decrease in systemic vascular resistance. These data indicate that ACh has potent vasodilator activity in the penile and systemic vascular beds.

The mechanism by which ACh induces vasodilation in the penis was investigated in experiments with the nonselective NOS inhibitor L-NAME and the nicotinic receptor antagonist hexamethonium. Following treatment with L-NAME the increase in ICP in response to ic injection of ACh was significantly attenuated suggesting that the release of NO from the endothelium in the corpora cavernosa is involved in mediating the response. The increase in ICP in response to ic injection of ACh was not altered by hexamethonium in a dose that attenuated the decrease in heart rate in response to preganglionic vagal stimulation suggesting that nicotinic receptor activation does not have a significant role in mediating or modulating the increase in ICP in response to the muscarinic receptor agonist. These results suggest that muscarinic receptor activation plays a significant role in mediating the erectile response to cavernosal nerve stimulation in the rat and that treatment with BAY 41-8543 would be useful in situations where cholinergic nerve function is impaired. The present results are consistent with results in the literature showing that the

erectile response to cavernosal nerve stimulation is reduced in the rat, dog and monkey by the administration of atropine^{24–28}. Senbel *et al.* (2011) recently reported that the inhibitory effect of atropine on the erectile response to cavernosal nerve stimulation in the rat was both dose- and stimulation frequency-dependent with inhibition of 5–25% (1–5 Hz) at the 1.5 mg/kg iv dose of atropine and 25–55% at the 10 mg/kg iv dose of atropine²⁷. The present data showing that responses to cavernosal nerve stimulation were attenuated by atropine are consistent with the results of Senbel *et al.* (2011) in the rat and support the concept that muscarinic receptor activation plays a significant role in mediating the penile erectile response and that cavernosal nerve stimulation releases NO and ACh. The present results are not in agreement with the studies in the literature showing that the response to nerve stimulation in the dog and rat is not altered by atropine^{29, 30}. Dail and colleagues reported that atropine had no effect on the response to cavernosal nerve stimulation in the rat, however, after lesion of the pelvic nerves were produced, erectile responses to nerve stimulation were attenuated by atropine³⁰. This explanation for the difference in results is uncertain, but does not involve specificity of the inhibitory effect of atropine for muscarinic receptors which has been suggested by some authors, since responses to ic injections of ACh were attenuated at a time responses to ic injection of SNP were not altered in the present study^{23, 31}.

Conclusion

In summary, results of the present study show that ic injections of the sGC stimulator BAY 41-8543 increase ICP/MAP and AUC in a dose-related manner in the anesthetized rat. The erectile response to BAY 41-8543 was synergistic with endogenously released and exogenously administered NO. The increase in ICP in response to cavernosal nerve stimulation was attenuated by atropine and restored by ic injection of BAY 41-8543. The erectile response to ic injection of ACh was attenuated by L-NAME and atropine, but was not altered by hexamethonium indicating that the response to ACh was mediated by the release of NO, the activation of muscarinic receptors in the corpora cavernosa and that nicotinic receptor activation does not play an important role. These data suggest that the activation of muscarinic receptors mediate approximately 35% of the increase in ICP in response to cavernosal nerve stimulation and indicate that the cavernosal nerves contain NO and ACh releasing terminals. BAY 41-8543 restored the erectile response to cavernosal nerve stimulation after cavernosal nerve crush injury. These data suggest that treatment with BAY 41-8543 would be useful in ED patients in which NO formation, release, or bioavailability are reduced by a disease process or when cholinergic or cavernosal nerve function are impaired by nerve injury.

Acknowledgments

These studies were supported by NIH grants HL62000 and HL77421.

References

1. Evgenov OV, Pacher P, Schmidt PM, Hasko G, Schmidt HH, Stasch JP. NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential. *Nat Rev Drug Discov.* 2006; 5:755–68. [PubMed: 16955067]
2. Mizusawa H, Hedlund P, Brioni JD, Sullivan JP, Andersson KE. Nitric oxide independent activation of guanylate cyclase by YC-1 causes erectile responses in the rat. *J Urol.* 2002; 167:2276–81. [PubMed: 11956492]
3. Stasch JP, Hobbs AJ. NO-independent, haem-dependent soluble guanylate cyclase stimulators. *Handb Exp Pharmacol.* 2009:277–308. [PubMed: 19089334]

4. Bischoff E, Schramm M, Straub A, Feurer A, Stasch JP. BAY 41–2272: a stimulator of soluble guanylyl cyclase induces nitric oxide-dependent penile erection in vivo. *Urology*. 2003; 61:464–7. [PubMed: 12597982]
5. Stasch JP, Alonso-Alija C, Apeler H, et al. Pharmacological actions of a novel NO-independent guanylyl cyclase stimulator, BAY 41–8543: in vitro studies. *Br J Pharmacol*. 2002; 135:333–43. [PubMed: 11815368]
6. Saenz de Tejada I, Angulo J, Celtek S, et al. Physiology of erectile function. *J Sex Med*. 2004; 1:254–65. [PubMed: 16422955]
7. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. *Circulation*. 2004; 109:III27–32. [PubMed: 15198963]
8. Bivalacqua TJ, Champion HC, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJ. Gene transfer of prepro-calcitonin gene-related peptide restores erectile function in the aged rat. *Biol Reprod*. 2001; 65:1371–7. [PubMed: 11673252]
9. Bivalacqua TJ, Champion HC, Purohit SK, et al. Nitric oxide-mediated erectile effects of galantide but not galanin in vivo. *Nitric Oxide*. 2000; 4:94–102. [PubMed: 10835289]
10. Champion HC, Bivalacqua TJ, Hyman AL, Ignarro LJ, Hellstrom WJ, Kadowitz PJ. Gene transfer of endothelial nitric oxide synthase to the penis augments erectile responses in the aged rat. *Proc Natl Acad Sci U S A*. 1999; 96:11648–52. [PubMed: 10500231]
11. Rask-Madsen C, King GL. Mechanisms of Disease: endothelial dysfunction in insulin resistance and diabetes. *Nat Clin Pract Endocrinol Metab*. 2007; 3:46–56. [PubMed: 17179929]
12. de Tejada IS. Therapeutic strategies for optimizing PDE-5 inhibitor therapy in patients with erectile dysfunction considered difficult or challenging to treat. *Int J Impot Res*. 2004; 16 (Suppl 1):S40–2. [PubMed: 15224136]
13. Padma-Nathan H, McCullough A, Forest C. Erectile dysfunction secondary to nerve-sparing radical retropubic prostatectomy: comparative phosphodiesterase-5 inhibitor efficacy for therapy and novel prevention strategies. *Curr Urol Rep*. 2004; 5:467–71. [PubMed: 15541217]
14. Angulo J, Gonzalez-Corrochano R, Cuevas P, et al. Diabetes exacerbates the functional deficiency of NO/cGMP pathway associated with erectile dysfunction in human corpus cavernosum and penile arteries. *J Sex Med*. 7:758–68. [PubMed: 19912487]
15. Shimokawa H. Primary endothelial dysfunction: atherosclerosis. *J Mol Cell Cardiol*. 1999; 31:23–37. [PubMed: 10072713]
16. Thomas GD, Zhang W, Victor RG. Nitric oxide deficiency as a cause of clinical hypertension: promising new drug targets for refractory hypertension. *JAMA*. 2001; 285:2055–7. [PubMed: 11311074]
17. Nakane M, Hsieh G, Miller LN, et al. Activation of soluble guanylate cyclase causes relaxation of corpus cavernosum tissue: synergism of nitric oxide and YC-1. *Int J Impot Res*. 2002; 14:121–7. [PubMed: 11979328]
18. Hsieh GC, O'Neill AB, Moreland RB, Sullivan JP, Brioni JD. YC-1 potentiates the nitric oxide/cyclic GMP pathway in corpus cavernosum and facilitates penile erection in rats. *Eur J Pharmacol*. 2003; 458:183–9. [PubMed: 12498924]
19. Fibbi B, Morelli A, Vignozzi L, et al. Characterization of phosphodiesterase type 5 expression and functional activity in the human male lower urinary tract. *J Sex Med*. 7:59–69. [PubMed: 19796053]
20. Hedlund P, Alm P, Andersson KE. NO synthase in cholinergic nerves and NO-induced relaxation in the rat isolated corpus cavernosum. *Br J Pharmacol*. 1999; 127:349–60. [PubMed: 10385233]
21. Spiess PE, Dion SB, Zvara P, Merlin SL, Chan PT, Brock GB. 7-Nitroindazole: a selective inhibitor of penile erection: an in vivo study in a rat animal model. *Urology*. 1996; 47:93–6. [PubMed: 8560670]
22. Mas M, Escrig A, Gonzalez-Mora JL. In vivo electrochemical measurement of nitric oxide in corpus cavernosum penis. *J Neurosci Methods*. 2002; 119:143–50. [PubMed: 12323418]
23. Andersson KE, Wagner G. Physiology of penile erection. *Physiol Rev*. 1995; 75:191–236. [PubMed: 7831397]
24. Stief C, Benard F, Bosch R, et al. Acetylcholine as a possible neurotransmitter in penile erection. *J Urol*. 1989; 141:1444–8. [PubMed: 2566691]

25. Stief C, Diederichs W, Benard F, et al. Possible role for acetylcholine as a neurotransmitter in canine penile erection. *Urol Int.* 1989; 44:357–63. [PubMed: 2576164]
26. Suh JK, Mun KH, Cho CK, Shin HC, Kim YS, Park TC. Effect of vasoactive intestinal peptide and acetylcholine on penile erection in the rat in vivo. *Int J Impot Res.* 1995; 7:111–8. [PubMed: 7496439]
27. Senbel AM, Hashad A, Sharabi FM, Daabees TT. Activation of muscarinic receptors inhibits neurogenic nitric oxide in the corpus cavernosum. *Pharmacol Res.*
28. Trigo-Rocha F, Hsu GL, Donatucci CF, Lue TF. The role of cyclic adenosine monophosphate, cyclic guanosine monophosphate, endothelium and nonadrenergic, noncholinergic neurotransmission in canine penile erection. *J Urol.* 1993; 149:872–7. [PubMed: 8384275]
29. Carati CJ, Creed KE, Keogh EJ. Autonomic control of penile erection in the dog. *J Physiol.* 1987; 384:525–38. [PubMed: 3656155]
30. Dail WG, Walton G, Olmsted MP. Penile erection in the rat: stimulation of the hypogastric nerve elicits increases in penile pressure after chronic interruption of the sacral parasympathetic outflow. *J Auton Nerv Syst.* 1989; 28:251–7. [PubMed: 2628468]
31. Larsson B, Andersson KE, Mattiasson A. Influence of antimuscarinics on alpha-adrenoceptors in the female rabbit urethra. *Acta Physiol Scand.* 1984; 120:537–42. [PubMed: 6148838]

n = 6-8

Figure 1.

Representative tracing showing changes in ICP, MAP, systolic and diastolic BP following injection of BAY 41-8543 100 µg/kg ic (A). Bar graphs showing change in ICP/MAP (B), AUC (C) and duration (D), in response to intracavernosal injection of BAY 41-8543 in the anesthetized rat. n indicates number of experiments, * indicates that the response is different than control value ($p < 0.05$).

n = 5-8

Figure 2.

Line graphs comparing changes in ICP (A) and MAP (B) in response to intracavernosal injection BAY 41-8543 and sodium nitroprusside. Bar graphs comparing the duration (C) and AUC (D) for the change in ICP in response to intracavernosal injections of BAY 41-8543 and sodium nitroprusside. n indicates number of experiments, * indicates $p < 0.05$ when responses to BAY 41-8543 and SNP are compared.

Figure 3.

Bar graphs showing the effect of atropine 1 mg/kg iv on change in ICP (A), ICP/MAP (B), and AUC (C) in response to cavernosal nerve stimulation at 2, 4, 8, 10 and 16 Hz (5V, 5 ms pulse, 60 sec stimulation period) in the anesthetized rat. n indicates number of experiments, * indicates $p < 0.05$ when compared to corresponding control.

Figure 4.

Bar graphs comparing changes in ICP in response to intracavernosal injections of acetylcholine 0.3 μ g/kg (A) and intracavernosal injection of sodium nitroprusside 0.3 μ g/kg (B) before and after administration of atropine 1 mg/kg iv. Bar graphs comparing changes in MAP in response to iv injection of acetylcholine 1 μ g/kg (C) and sodium nitroprusside 3 μ g/kg (D) before and after administration of atropine 1 mg/kg iv. n indicates number of experiments, $p < 0.05$ when compared with corresponding control.

Figure 5.

Bar graphs showing the effects of injections of acetylcholine 1 $\mu\text{g/kg}$ iv on systemic arterial pressure (A), cardiac output (B), and heart rate (C) before (open bars) and after (black bars) treatment with atropine 1 mg/kg iv. n indicates number of experiments, * indicates $p < 0.05$ when compared to control.

Figure 6.

Bar graphs comparing changes in ICP, ICP/MAP, and AUC in response to intracavernosal injections of acetylcholine (0.3 µg/kg) before and after injections hexamethonium (2.5mg/kg iv) (A) and L-NAME (50mg/kg iv) (B). n indicates number of experiments, * indicates $p < 0.05$ when compared to controls.

Figure 7.

Bar graphs comparing change in ICP (A), ICP/MAP (B), and AUC (C) in response to cavernosal nerve stimulation at 4 Hz before and after administration of atropine 1 mg/kg iv and BAY 41-8543 0.1 µg/kg ic. n indicates number of experiments, * indicates $p < 0.05$ when compared to response at 4 Hz.

Figure 8.

Bar graphs comparing increase in ICP (A) in response to separate ic injection of sodium nitroprusside 0.01 $\mu\text{g/kg}$ and BAY 41-8543 0.3 $\mu\text{g/kg}$, the algebraic sum of individual responses to sodium nitroprusside and BAY 41-8543 and the response to combination injection of sodium nitroprusside and BAY 41-8543 from the same syringe and bar graphs comparing the area under the curve for the algebraic sum of individual responses to ic injection of sodium nitroprusside and BAY 41-8543 and the response to combination injection with the same syringe.

Bar graphs comparing increase in ICP (B) in response to ic injection of BAY 41-8543 0.1 $\mu\text{g/kg}$ and cavernosal nerve stimulation at 2 Hz, the algebraic sum of responses to ic BAY 41-8543 injection and nerve stimulation at 2 Hz and the combined response to nerve stimulation and ic injection of BAY 41-8543. Bar graphs comparing the area under the curve for the algebraic sum of individual responses to stimulation at 2 Hz and ic injection of BAY 41-8543 and the response to the combined stimuli. n indicates number of experiments, * indicates $p < 0.05$ when the combined response to BAY 41-8543 and nerve stimulation is compared to the algebraic sum of individual responses to BAY 41-8543 and cavernosal nerve stimulation.

Figure 9.

Representative tracing from a cavernosal nerve crush injury experiment in which the increase in ICP in response to nerve stimulation (10 Hz) was measured before and after nerve crush and BAY 41-8543 was injected (A). Bar graphs showing the time course of the effect of acute crush of the cavernosal nerve on the responses to cavernosal nerve stimulation (n = 3) (10 Hz) (B). n indicates number of experiments, * indicates $p < 0.05$ when compared to control.

Bar graphs comparing the change in ICP (C), ICP/MAp (D), and AUC (E) in response to cavernosal nerve stimulation (10Hz) in control (open bar), following nerve crush (filled bar), and following nerve crush with simultaneous administration of BAY 41-8543 (1µg/kg ic). n indicates number of experiments, * indicates $p < 0.05$ when compared to control.

Decrease in MAP at Time of Maximum ICP and Peak Decrease in MAP and time of occurrence in response to intracavernosal injections of Bay 41-8543

Table 1

Bay 41-8543(μ g/kg ic)	Decrease in MAP at Max ICP (mmHg)	Time Occurred (sec)	Peak Decrease in MAP (mmHg)	Time Occurred (sec)
0.1	-1.3 ± 0.5	22 ± 3	-1.5 ± 0.7	56 ± 11
0.3	$-1.6 \pm 0.3^*$	34 ± 2	-3.6 ± 0.9	105 ± 15
1	$-2 \pm 0.9^*$	37 ± 3	-5.4 ± 1.6	126 ± 14
3	$-2.5 \pm 0.5^*$	47 ± 5	-8 ± 0.4	163 ± 29
10	$-7.8 \pm 2.2^*$	50 ± 7	-13.3 ± 3.5	201 ± 17
30	$-14.4 \pm 3.7^*$	59 ± 8	-27.9 ± 3.8	207 ± 27
100	$-22 \pm 3.9^*$	90 ± 29	-50 ± 3.3	223 ± 36

Values are expressed as mean $\pm$ SE.

* denotes a P value < 0.05 when compared to control.