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Characterization of a novel phosphodiesterase type 5 inhibitor: JNJ-10258859

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Abstract

We have characterized a novel, potent, and selective phosphodiesterase type 5 inhibitor, JNJ-10258859 ((*R*)-(−)-3-(2,3-dihydro-benzofuran-5-yl)-2-[5-(4-methoxy-phenyl)-pyrimidin-2-yl]-1,2,3,4-tetrahydro-pyrrolo[3,4-b]quinolin-9-one). Its inhibitory effects on phosphodiesterase 1–6 were determined using enzymes partially purified from human tissues. The compound inhibited phosphodiesterase type 5 with a K_i of 0.23 nM and displayed excellent selectivity versus phosphodiesterase types 1–4 (≥22,000 fold compared to phosphodiesterase type 5). It had 27-fold selectivity over phosphodiesterase type 6 as well. In a cell-based assay, JNJ-10258859 was more potent than sildenafil in potentiating nitric oxide (NO) induced accumulation of intracellular cGMP. The in vivo effect of JNJ-10258859 was evaluated in an anesthetized dog model via intravenous administration. The compound had similar efficacy to sildenafil in enhancing both the amplitude and duration of intracavernosal pressure increase induced by electrical stimulation to the pelvic nerve. No significant effects on either mean aortic pressure or heart rate were observed during the course of the experiments. This data suggests that JNJ-10258859 could be a useful treatment for erectile dysfunction.

Keywords: Phosphodiesterase; Phosphodiesterase type 5 inhibitor; Erectile dysfunction; Corpus cavernosum

1. Introduction

Male erectile dysfunction is a condition that affects the quality of life of many men. The Massachusetts Male Aging Study, the first large-scale comprehensive epidemiological study of erectile dysfunction, found that 52% of a population of men age 40–70 years old suffered from the condition. Of those, 33% had minimal erectile dysfunction, 48% had moderate erectile dysfunction and 19% had complete erectile dysfunction (Feldman et al., 1994).

It is well established that normal erection is primarily mediated through the nitric oxide (NO)/cGMP pathway (Ignarro et al., 1990; Kim et al., 1991; Burnett et al., 1992). Upon sexual stimulation, nitric oxide is released from non-cholinergic non-adrenergic neurons and the endothelium. This release of NO activates soluble guanylyl cyclases in the cavernosal muscles and associated arterioles to catalyze the conversion of GTP to cGMP. Elevated cGMP levels trigger a cascade of down-stream events leading to relaxation of the smooth muscle cells lining the sinusoids and the arterioles that empty into them. This results in increased blood flow into the sinusoids and distention of the cavernosal bodies. The engorged cavernosal bodies block the venous out flow leading to pressure build up in the *corpora* and a rigid erection. The accumulated cGMP is mainly degraded by phosphodiesterase type 5 in cavernosal smooth muscle (Boolell et al., 1996). In most erectile dysfunction patients, the amount of NO released is insuffi-

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cient to cause an adequate accumulation of cGMP necessary for a complete relaxation. Inhibition of phosphodiesterase type 5 activity potentiates cGMP accumulation and facilitates an erection.

Phosphodiesterase type 5 is a member of the phosphodiesterase family. Phosphodiesterases modulate the levels and duration of actions of cyclic nucleotides via their ability to hydrolyze cAMP and/or cGMP.

To date, 21 mammalian phosphodiesterase genes have been cloned and classified into 11 families according to the sequence homology and biochemical properties. These families are: phosphodiesterase type 1, Ca^{2+}/calmodulin dependent; phosphodiesterase type 2, cGMP-stimulated; phosphodiesterase type 3, cGMP-inhibited; phosphodiesterase type 4, cAMP-specific and rolipram-sensitive; phosphodiesterase type 5, cGMP-specific; phosphodiesterase type 6, photoreceptor cGMP-specific; phosphodiesterase type 7, cAMP-specific and rolipram-insensitive; phosphodiesterase type 8, cAMP-specific and IBMX-insensitive; phosphodiesterase type 9, cGMP-specific; phosphodiesterase type 10 and phosphodiesterase type 11, hydrolyzing both cAMP and cGMP (Francis et al., 2001). Most of the phosphodiesterases are expressed in multiple tissues and multiple phosphodiesterases may co-exist in any given tissue. Nevertheless, some phosphodiesterases show differential expression patterns, making partial tissue-specific inhibition of phosphodiesterase activity feasible. Sildenafil, a phosphodiesterase type 5 selective inhibitor, is a particularly relevant proof of this principle (Boolell et al., 1996).

The introduction of sildenafil as an oral agent for the treatment of erectile dysfunction sparked tremendous interest in the search for next generation phosphodiesterase type 5 inhibitors with a better selectivity profile. Although sildenafil is very efficacious in treatment of mild to moderate erectile dysfunction, its limited selectivity against phosphodiesterase types 1 and 6 may be responsible for some of the side effects reported to date (Eardley, 1997). Here, we report the characterization of JNJ-10258859 ((*R*)-(−)-3-(2,3-dihydro-benzofuran-5-yl)-2-[5-(4-methoxy-phenyl)-pyrimidin-2-yl]-1,2,3,4-tetrahydro-pyrrolo[3,4-b]quinolin-9-one) (Fig. 1 and Sui et al., 2002), a novel phosphodiesterase type 5 inhibitor, with a selectivity profile better than sildenafil. JNJ-10258859 is efficacious in potentiating an increase in intracavernosal pressure in an anesthetized dog model measuring erectile status.

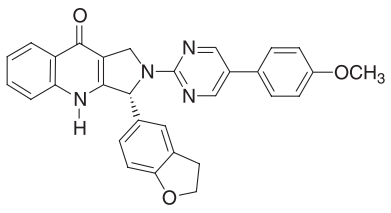

Fig. 1. Structure of JNJ-10258859, (*R*)-(−)-3-(2,3-dihydro-benzofuran-5-yl)-2-[5-(4-methoxy-phenyl)-pyrimidin-2-yl]-1,2,3,4-tetrahydro-pyrrolo[3,4-b]quinolin-9-one.

2. Materials and methods

2.1. Materials

Human tissue homogenates were obtained from Analytical Biological Services, (Wilmington, DE). [^{3}H]cGMP and [^{3}H]cAMP were from Amersham Pharmacia Biotech (Piscataway, NJ). Sildenafil was extracted from Viagra® tablets and used as the citrate salt. JNJ-10258859 was synthesized as previously described (Sui et al., 2002). All the other chemicals were purchased from Sigma unless otherwise indicated.

2.2. Methods

2.2.1. Phosphodiesterase isolation and assay

Human corpus cavernosum, platelet, skeletal muscle, heart, and retina homogenates were obtained and enzyme preparations carried out essentially as previously described (Qiu et al., 2000) with minor modifications on the elution gradient. A linear gradient of 100–500 mM NaCl in 45 column-volume was used for corpus cavernosum, 100–600 mM in 60 column-volume for platelet, 200–500 mM in 35 column-volume for skeletal muscle, 150–550 mM in 50 column-volume for heart, and 150–550 mM in 45 column-volume for retina. The elution profile was analyzed using a phosphodiesterase assay carried out essentially as described by Thompson and Appleman (1971) with minor modifications to adapt the assay to a 96-deep well format. Briefly, the enzyme was assayed in 5 mM $MgCl_2$, 15 mM Tris HCl (pH 7.4), 0.5 mg/ml bovine serum albumin, 1 μM cAMP or cGMP and 0.1 μCi [^{3}H]cGMP or [^{3}H]cAMP. The total volume of the assay was 100 μl. The reaction mixture was incubated at 30 °C for 30 min and then stopped by boiling for 1 min followed by cooling on ice. The resulting [^{3}H] 5′-mononucleotides were further converted to uncharged [^{3}H] nucleosides by adding 25 μl 1 mg/ml snake venom (*Ophiophagus hannah*) and incubating at 30 °C for 10 min. This reaction was stopped by the addition of 1 ml AG1-X2 resin slurry (Bio-Rad Laboratories, Hercules, CA). After centrifugation, a 200-μl aliquot of supernatant was quantified by liquid scintillation. Phosphodiesterase activity was expressed as pmol of cyclic nucleotide hydrolyzed per min per ml of column elute. The amount of elution used was such that the hydrolysis of substrates in the peak fractions did not exceed 15% of the total, ensuring that the amount of product increased linearly with respect to time.

2.2.2. Inhibitory effects of JNJ-10258859 and sildenafil

Phosphodiesterase types 1, 5 and 6 were assayed with cGMP as the substrate and phosphodiesterase types 2, 3 and 4 were assayed with the cAMP as substrate. The phosphodiesterase assay was run essentially as described above, except that 30 nM [^{3}H]cGMP or [^{3}H]cAMP was used for substrate and the incubation time was 90 min. For compound inhibition studies on the phosphodiesterases, a stock solution

of the test compound was prepared and diluted in 100% dimethyl sulfoxide (DMSO) to the appropriate concentration, and added to the assay buffer to give a final concentration of 2% DMSO. The amount of enzyme used in each reaction was such that the hydrolysis of substrates did not exceed 15% of the total, ensuring that the amount of product increased linearly with respect to time. Duplicate experiments were done in each assay. Percent inhibition was used to generate IC_{50} values by fitting the data to a nonlinear regression model in GraphPad Prism (San Diego, CA). At very low concentrations of substrate ([S]≪Km), these IC_{50} values approximate the K_i values.

2.2.3. RFL-6 cell assay

Rat fetal lung fibroblast (RFL-6) cells (passage number 4 from ATCC frozen stock) were grown to confluence in 48-wells plates. The culture medium was removed and the cells were washed twice with 1.0 ml Locke's buffer (NaCl, 154.0 mM; KCl, 5.6 mM; $CaCl_2$, 2.0 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 3.6 mM; glucose, 5.6 mM; HEPES, 10 mM, pH 7.4). The cell monolayers were then equilibrated for 1 h at 37 °C in 0.25 ml of Locke's buffer. Following the 1-h incubation, the buffer was removed and 170 μl of Locke's buffer containing either the phosphodiesterase inhibitor (dissolved in 100% DMSO) or vehicle was added to the cells to generate a final DMSO concentration of was 0.35%. The cells were incubated for an additional 15 min at 37 °C and then freshly prepared sodium nitroprusside was added to reach a final concentration of 6 μM. The cells were incubated for an additional 10 min. The medium was removed and the cells were washed with 0.5 ml fresh Locke's buffer. The cells were lysed with reagent 1 from the SPA kit for cAMP (Amersham Pharmacia Biotech).

The cAMP SPA direct screening assay system (Amersham Pharmacia Biotech) was modified to determine the intracellular levels of acetylated cGMP. Reagents from the cGMP tube-based SPA assay (Amersham Pharmacia Biotech) were diluted and handled using the same procedures as for the cAMP direct screening assay. Lysis reagent 2 from the SPA kit for cAMP was used to dilute the trace, antiserum and SPA reagent components of the cGMP kits (15 ml per vial). The acetylated cyclic nucleotides were diluted in lysis reagent 1 to give the desired concentration range for standard curve generation. cGMP levels were determined in the lysates according to the manufacturer's assay procedure. The data were analyzed by nonlinear regression analysis using the statistical program GraphPad Prism. Each dose was assayed in triplicate wells within an experiment. Cyclic nucleotide content in each well was determined in duplicate. The cGMP levels induced by 6 μM sodium nitroprusside was designated as baseline. Results were expressed as the fold increase in cGMP levels over baseline.

2.2.4. In vivo efficacy study

All animals were handled in accordance with the NRC *Guide for Care and Use of Laboratory Animals* and the protocol was approved by Internal Animal Care and Usage Committee of Johnson and Johnson Pharmaceutical Research and Development, LLC. An anesthetized dog model was established with modifications from those described in the literature (Vardi et al., 1987; Carter et al., 1998). Briefly, Male beagles (8–15 kg) were fasted overnight. The animals were anesthetized by sodium pentobarbital injection (35 mg/kg) through the cephalic vein. Anesthesia was maintained throughout the course of the experiment by continuous infusion of pentobarbital (4 mg/kg/h) through the left femoral vein (Harvard Apparatus, Holliston, MA 01745). The dog was placed on a respirator and the body temperature maintained at 36–38 °C (TP500 Hydrothermic System, Kent Scientific, Litchfield, CT 06759). Catheters were introduced into the right femoral artery and vein for recording systemic arterial blood pressure and compound delivery, respectively. A mid-line abdominal incision was made and bladder completely emptied. The pelvic nerve plexus was identified, dissected free from surrounding tissues, and placed into a subminiature electrode. The penis was denuded of skin down to the base. A 19-gauge needle attached by a flexible catheter to a pressure transducer was inserted into the corpus cavernosum to record intracavernosal pressure (ICP) changes in response to electrical stimulation of the pelvic nerve. Parameters were recorded on Crystal Biotech CBI-8000 (GOULD Instrument System, Valley View, OH) and data was acquired through a PONEMAH P3 (GOULD Instrument System) series. Pelvic nerve was stimulated with a Grass S88K square pulse stimulator and a CCU1 constant current unit (Grass, West Warwick, RI 02893). A current output was set at 15–30 mA, 3-ms pulse duration, 1–10 Hz frequency range, and 1–2 min stimulation.

After a period of stabilization, the control intracavernosal pressure response curves were generated at an appropriate current output setting that induced intracavernosal pressure increases between 20% and 30% of systolic pressure. When similar increases in intracavernosal pressure were obtained from at least two control stimulations applied in 15-min interval, the control response was established. Area under the curve (AUC) was computed and designated as baseline. Compounds were dissolved in 10% solutol prepared in 5% dextrose. Ascending doses of compound were administrated via bolus injection to the right femoral vein at 40-min interval. The effect of compound on the increase in intracavernosal pressure was evaluated 15 min after each dosing by electrical stimulation with the frequency setting the baseline. To quantify the effect of compound, AUC for the intracavernosal pressure curve at each post dosing stimulation was computed and the baseline AUC subtracted. At the end of each experiment, 300 μg/kg sildenafil was given the same way as the testing compound. This dose was shown to induce a maximal response in our hands. For each animal, the highest AUC increase was designated as 100%. The EC_{50} was generated with a nonlinear regression curve-

fitting model. The values of heart rate and mean aortic blood pressure before compound administration were designated as baselines. Before each efficacy evaluation, the heart rate and mean arterial pressure were compared with the baseline and the percentage change over the baseline values calculated.

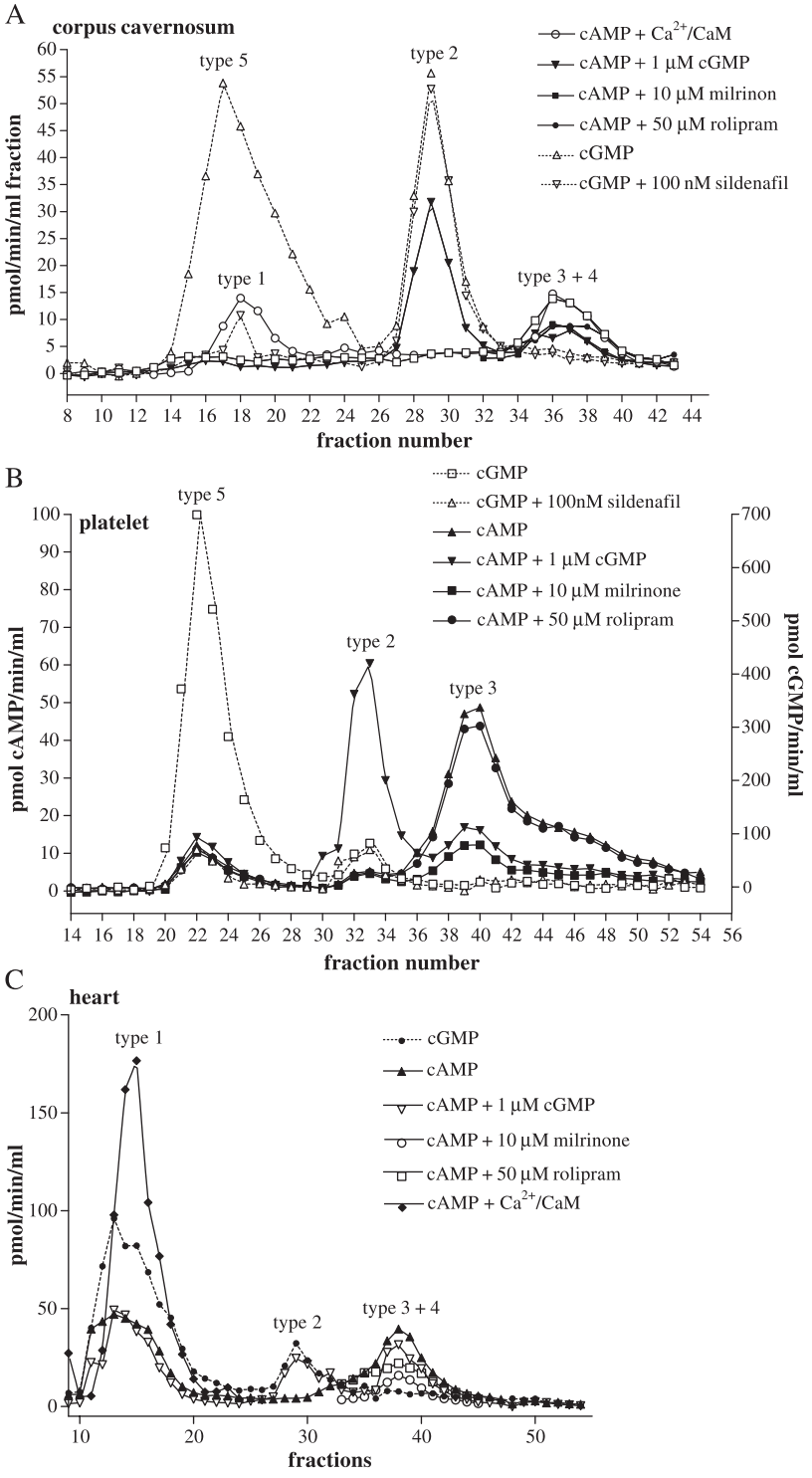

Fig. 2. Characterization of cAMP and cGMP phosphodiesterase activities in elution fractions from human corpus cavernosum (A), platelet (B) and heart (C). Selective activator/inhibitors were used to identify different phosphodiesterases: Ca^{2+}/CaM stimulation for phosphodiesterase type 1, cGMP stimulation for phosphodiesterase type 2, milrinone inhibition for phosphodiesterase type 3, rolipram inhibition for phosphodiesterase type 4 and sildenafil inhibition for phosphodiesterase type 5. One μM cAMP or cGMP was used as substrate. Representative data from at least four studies.

3. Results

3.1. Phosphodiesterases isolation

Phosphodiesterase types 2 and 5 were partially purified from human corpus cavernosum. The elution profile showed there were two major peaks with cGMP hydrolyzing activity and one with cAMP hydrolyzing activity (Fig. 2A). The first peak mainly contained phosphodiesterase type 5 as judged by its sensitivity to 100 nM sildenafil. A component with some phosphodiesterase type 1 activity co-eluted with phosphodiesterase type 5 in the first peak since Ca^{2+}/calmodulin stimulated the cAMP hydrolyzing activity in this peak. The second peak had cGMP hydrolyzing activity and its cAMP hydrolyzing activity could be stimulated by cGMP, indicating the presence of phosphodiesterase type 2. Third peak only consisted of cAMP hydrolyzing activity that was sensitive to rolipram, milrinone and cGMP, suggesting the presence of both phosphodiesterase type 3 and phosphodiesterase type 4.

Phosphodiesterase type 3 was isolated from human platelet (Fig. 2B). The elution profile of the platelet homogenate was very similar to that of the corpus cavernosum, especially for peaks 1 and 2, containing mainly phosphodiesterase types 5 and 2, respectively. Thus, phosphodiesterase types 2 and 5 could also be obtained from platelets. The third peak showed cAMP hydrolyzing activity only. The activity was insensitive to rolipram and sensitive to milrinone and cGMP, indicating the majority of enzyme activity in this peak was contributed by phosphodiesterase type 3. Ca^{2+} and calmoldulin did not stimulate any increase in cAMP hydrolyzing activity (data not shown).

Phosphodiesterase type 1 was obtained from human heart tissue with three peaks in the elution profile (Fig. 2C). The first peak contained both cAMP and cGMP hydrolyzing activity. Its activity could be stimulated by Ca^{2+} and calmoldulin, indicating the presence of phosphodiesterase type 1. The cAMP hydrolyzing activity in peak 2 was stimulated by cGMP, suggesting the existence of phosphodiesterase type 2. The third peak consisted of both phosphodiesterase types 3 and 4 since it was sensitive to both milrinone and rolipram.

Table 1
Inhibitory effect of JNJ-10258859 and sildenafil on human phosphodiesterase types 1–6

Phosphodiesterase	Sildenafil		JNJ-10258859	
	$K_i \pm$ S.D.	Ratio[a]	$K_i \pm$ S.D.	Ratio[a]
Type 5	1.70 ± 0.28 nM	1	0.23 ± 0.03 nM	1
Type 1	0.28 ± 0.07 μM	160	>100 μM	>434,000
Type 2	13.11 ± 3.34 μM	7000	25.33 ± 5.53 μM	110,000
Type 3	14.46 ± 0.81 μM	8500	9.67 ± 1.95 μM	42,000
Type 4	6.48 ± 0.80 μM	3800	5.01 ± 1.88 μM	22,000
Type 6_{cone}	16.63 ± 1.48 nM	8	6.20 ± 1.42 nM	27
Type 6_{rod}	20.60 ± 0.78 nM	12	6.72 ± 0.76 nM	29

$N = 3–4$.
[a] K_i of phosphodiesterase/K_i of phosphodiesterase type 5.

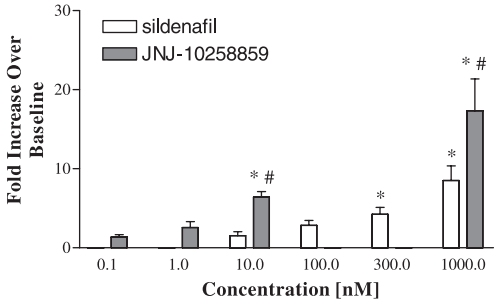

Fig. 3. Effect on sodium nitroprusside induced cGMP accumulation in RFL-6 cells. RFL-6 cells were treated with various concentrations of sildenafil or JNJ-10258859, followed by 6 μM sodium nitroprusside. The amounts of cGMP generated were expressed as fold increase over a baseline set by 6 μM sodium nitroprusside treatment alone. $N = 4–7$ except for JNJ-10258859 at 1 μM where $n = 3$. Error bar represents standard deviation. $^{*}P < 0.001$ versus baseline by one-way ANOVA. $^{\#}P < 0.01$ versus sildenafil at the same concentrations by Student's t-test.

Phosphodiesterase type 4 was partially purified from human skeletal muscle (data not shown). Phosphodiesterase type 6 was isolated from human retina (data not shown). There were two peaks with cGMP hydrolyzing activity; the minor peak containing cone phosphodiesterase type 6 preceded the major peak with rod phosphodiesterase type 6 (Gillespie and Beavo, 1988). The identity of phosphodiesterase type 6 was confirmed by trypsin activation of the enzyme (data not shown).

3.2. Inhibitory effects of JNJ-10258859 on various phosphodiesterases

The inhibitory effect of the compound was tested against the phosphodiesterases partially purified from human tissues as described above (Table 1). Sildenafil was used as a benchmark and tested in parallel in every assay. JNJ-10258859 was a very potent phosphodiesterase type 5 inhibitor, with a K_i of 0.23 nM, seven times more potent than sildenafil (1.7 nM). It was essentially inactive on phosphodiesterase type 1 since at 100 μM less than 50% inhibition was observed. In contrast, sildenafil showed a K_i

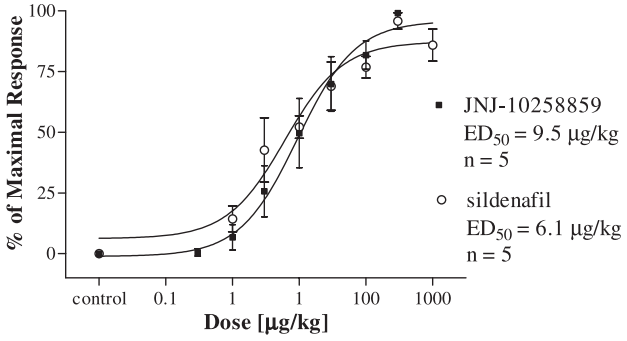

Fig. 4. Effect of JNJ-10258859 and sildenafil on intracavernosal pressure in anesthetized dogs. Ascending doses of compound were delivered intravenously at 40-min intervals. Effects were evaluated at 15 min post dosing. Bar represents standard deviation from n of 5.

of 280 nM on phosphodiesterase type 1. A selectivity of 27- and 29-fold was achieved for cone and rod phosphodiesterase type 6, respectively, as compared to 8- and 12-fold for sildenafil. JNJ-10258859 showed more than 22,000-fold selectivity over phosphodiesterase types 2, 3 and 4.

3.3. Effect of JNJ-10258859 on cGMP accumulation in RFL-6 cells

To determine whether JNJ-10258859 could penetrate cell membrane and inhibit phosphodiesterase type 5 activity inside the cells, sodium nitroprusside induced cGMP accumulation was investigated in RFL-6 cells in the presence and absence of JNJ-10258859. Sildenafil was also tested for comparison. Because of its increased potency against phosphodiesterase type 5, JNJ-10258859 was tested at lower concentrations than sildenafil. Both compounds showed dose-dependent potentiation of intracellular cGMP concentrations (Fig. 3). At 10 nM, sildenafil induced a 1.53-fold increase on intracellular cGMP concentrations, while JNJ-10258859 elicited a 6.4-fold increase.

3.4. Effect of JNJ-10258859 in an efficacy model

The efficacy of JNJ-10258859 was tested in an anesthetized dog model. The ability of JNJ-10258859 to potentiate an increase in intracavernosal pressure (ICP) induced by electric stimulation to the pelvic nerve was evaluated. Ascending doses were administrated intravenously to each dog. Sildenafil was tested in the same model. As shown in Fig. 4, both compounds potentiated a dose-dependent increase in intracavernosal pressure; JNJ-10258859 was as potent as sildenafil. The heart rate and mean aortic blood pressure were continuously monitored during the course of the experiment. The values of these two parameters observed immediately before each efficacy evaluation were compared to the values prior to initial dosing. Neither JNJ-10258859 nor sildenafil induced statistical significant changes on heart rate or mean aortic pressure (Fig. 5).

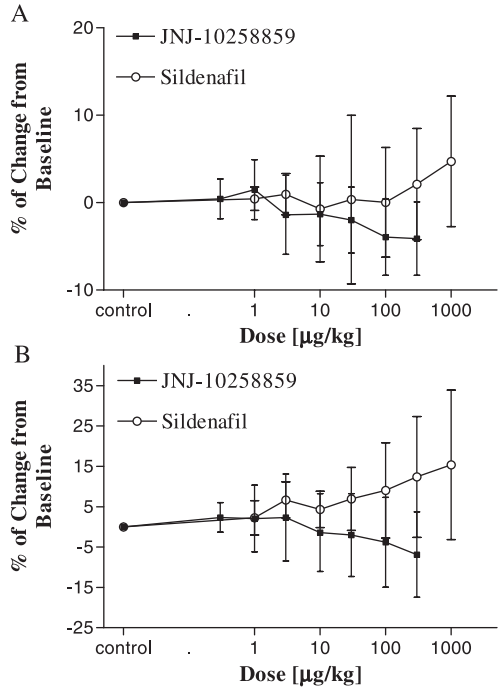

Fig. 5. Effect of JNJ-10258859 and sildenafil on mean aortic pressure (A) and heart rate (B) in anesthetized dogs. The values observed right before efficacy evaluation were compared with those prior to dosing (baseline). Bar represents standard deviation from *n* of 5.

4. Discussion

Since JNJ-10258859 was evaluated with phosphodiesterase types 1–6 partially purified from human tissues, it was important to confirm the identity of these phosphodiesterases during isolation to ensure proper characterization of the compound. In corpus cavernosum preparation, phosphodiesterase type 5 activity could be easily separated from other phosphodiesterases. The small amount of phosphodiesterase type 1 that co-eluted with phosphodiesterase type 5 was negligible and did not have significant effect on phosphodiesterase type 5 activity. Phosphodiesterase type 2 was also well separated from the other phosphodiesterases, while phosphodiesterase types 3 and 4 co-eluted. It was reported by Boolell et al. (1996) that human corpus cavernosum had phosphodiesterase types 2, 3 and 5 activity, while Taher et al. (1997) described phosphodiesterase types 3, 4 and 5 activity. We consistently observed phosphodiesterase types 1, 2, 3, 4 and 5 in human corpus cavernosum from four different cadaver donors. The mRNA of phosphodiesterase types 1, 2, 3, 4, 5, 7, 8, and 9 was detected in human corpus cavernosum by reverse transcription-polymerase chain reaction (RT-PCR) (Kuthe et al., 2001). Although the lack of selective inhibitors precluded individual examination of phosphodiesterase types 7–9 activities in our preparation, the activities displayed by selective inhibitors of phosphodiesterase types 2–5 indicated that there was no significant presentation of phosphodiesterase types 7–9 in the human corpus cavernosal tissues used in our experiments.

Platelets are a rich source of phosphodiesterase type 5 (Hamet and Coquil, 1978; Wallis et al., 1999). Our platelet preparation was consistent with these literature reports and showed extremely high phosphodiesterase type 5 activity. In addition, phosphodiesterase types 2 and 3 activities were also evident. Although phosphodiesterase type 1 often co-eluted with phosphodiesterase type 5 in other tissues (Shahid et al., 1991; Torphy et al., 1993; Qiu et al., 2000), the cAMP hydrolyzing activity that co-eluted with platelet phosphodiesterase type 5 was not phosphodiesterase type 1 since it was insensitive to Ca^{2+} and calmoldulin stimulation. The identity of this activity remains to be established. Rolipram did not exhibit inhibitory effect on cAMP hydrolyzing activity, suggesting that phosphodies-

terase type 4 was not present in platelet preparation. Indeed, platelets were the only source among the tissues we surveyed whose phosphodiesterase type 3 activity was not contaminated by that of phosphodiesterase type 4. As previously reported, the major phosphodiesterase in human cardiac muscle was phosphodiesterase type 1 (Wallis et al., 1999). It was also observed that phosphodiesterase types 2, 3 and 4 were present in the preparation. Using these partially purified phosphodiesterases, we characterized the inhibitory effects of JNJ-10258859 on phosphodiesterase types 1–6. Sildenafil was always used as a control and the data generated are in good agreement of existing literature (Boolell et al., 1996).

Since the launch of Viagra, more than a dozen new phosphodiesterase type 5 inhibitors have been reported. Several of them have IC_{50} values in the single digit nM range (Coste and Grondin, 1995; Silver et al., 1998; Kotera et al., 2000; Hosogai et al., 2001; Sorbera et al., 2001). Among the phosphodiesterase type 5 inhibitors with published data, vardenafil is most potent against phosphodiesterase type 5, with an IC_{50} of 0.7 nM (Saenz de Tejada et al., 2001). In our assay systems, JNJ-10258859 has potency comparable to the literature values of vardenafil on phosphodiesterase type 5.

JNJ-10258859 is also a selective phosphodiesterase type 5 inhibitor. Compared to sildenafil and vardenafil, it has significantly better selectivity versus phosphodiesterase type 1. Less than 50% of phosphodiesterase type 1 activity was inhibited at 100 μM concentrations of JNJ-10258859, while sildenafil and vardenafil have phosphodiesterase type 1/type 5 ratios of 160 and 257 (Saenz de Tejada et al., 2001), respectively. Although the side effects observed in sildenafil usage, such as headache, flushing, dyspepsia and rhinitis, are probably caused by phosphodiesterase type 5 inhibition itself, involvement of phosphodiesterase type 1, which is expressed in heart and vascular smooth muscles, has not been completely ruled out. Another frequently reported side effect of sildenafil usage is visual disturbance, caused by the cross inhibition of phosphodiesterase type 6, the critical enzyme involved in visual signal transduction, by sildenafil. Since JNJ-10258859 exhibits phosphodiesterase type 6 selectivity 2- to 3-fold better than sildenafil, fewer visual side effects are expected. By comparison, no visual side effect was observed in clinical trials of vardenafil (Ormrod et al., 2002), whose phosphodiesterase type 6 selectivity is also 2-fold better than sildenafil (Saenz de Tejada et al., 2001).

To assess the ability of JNJ-10258859 to inhibit phosphodiesterase type 5 inside living cells, we chose the RFL-6 cells since it has been shown that RFL-6 cells have an intact NO/sGC pathway (Schroder et al., 1987). We characterized the phosphodiesterase profile of the RFL-6 cells and demonstrated that phosphodiesterase type 5 is the major cGMP-hydrolyzing enzyme (unpublished), indicating its suitability as a model system for the evaluation of phosphodiesterase type 5 inhibitors inside the cells. The results showed that JNJ-10258859 was efficacious in potentiating cGMP accumulation in RFL-6 cells, demonstrating its ability to penetration cell membrane. Furthermore, it was more potent than sildenafil in this assay system, a result consistent with the improved potency of JNJ-10258859 on isolated phosphodiesterase type 5.

An anesthetized dog model was used to evaluate the in vivo effects of our phosphodiesterase type 5 inhibitor. To better compare compounds, it was important to obtain an EC_{50} value for each compound. We observed, as others have (Carter et al., 1998), that the extent of the increase in intracavernosal pressure in response to a set frequency of electric stimulation to the pelvic nerve varied considerably from animal to animal. It was difficult to compare the absolute values of intracavernosal pressure increases obtained from different animals even when an identical dose of compound was administrated. To address these issues, we administered increasing doses of compound to each dog intravenously. At the end of the experiment, a large dose of sildenafil (300 μg/kg), previously determined to induce maximal response, was delivered to calibrate the maximal response that could be reached for each dog. The addition of sildenafil did not further increase the response produced by JNJ-10258859, suggesting maximal effect was reached with this compound. Although JNJ-10258859 was more potent than sildenafil in enzyme assay and in RFL-6 cell based assay, it had potency similar to sildenafil in vivo. A possible explanation is that the two compounds have different plasma protein binding. For sildenafil, 86% drug was bound to plasma protein (Walker et al., 1999). Although it was not yet been experimentally determined, higher plasma protein binding for JNJ-10258859 could cause the amount of free drug to decrease significantly.

After exposure to increasing doses of JNJ-10258859, the dogs showed a trend of decreasing mean aortic pressure, though its effect was not statistically significant. After sildenafil treatment, the mean aortic pressure increased slightly at high doses, but this was not mechanistically based since cGMP accumulation would have the opposite effect. The heart rate showed a trend of dose-dependent increase after sildenafil treatment, but the data were not statistically significant. Dogs dosed with JNJ-10258859 exhibited non-mechanistically based decreases in heart rate, which again was statistically insignificant. Thus, the two compounds could not be differentiated on their ability to perturb heart rate and mean aortic pressure in this model.

In summary, we have identified a novel phosphodiesterase type 5 inhibitor, JNJ-10258859, that was more potent and selective than sildenafil. It was more active than sildenafil in potentiating NO induced cGMP accumulation in a cell-based assay and as efficacious as sildenafil in an anesthetized dog model. JNJ-10258859 could be a useful tool in studying the functions of phosphodiesterase type 5 in diverse physiological processes and as well as a potential treatment for erectile dysfunction.

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