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FR226807: a potent and selective phosphodiesterase type 5 inhibitor

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Abstract

We describe the pharmacological characteristics of a novel phosphodiesterase type 5 inhibitor FR226807, *N*-(3,4-dimethoxybenzyl)-2-{[(1*R*)-2-hydroxy-1-methylethyl]amino}-5-nitrobenzamide. FR226807 inhibited phosphodiesterase type 5 isolated from human platelets with an IC_{50} value of 1.1 nM. FR226807 also inhibited phosphodiesterase type 6 with an IC_{50} of 20 nM; however, the IC_{50} value for phosphodiesterase type 6 was 18-fold higher than that for phosphodiesterase type 5. The IC_{50} values of FR226807 for other phosphodiesterases (phosphodiesterase type 1, phosphodiesterase type 2, phosphodiesterase type 3, and phosphodiesterase type 4) were 1000-fold higher than that for phosphodiesterase type 5. FR226807 increased the cyclic guanosine monophosphate (cGMP) content in corpus cavernosum isolated from rabbit, an effect associated with relaxation of the muscle. FR226807 enhanced the relaxation response induced by electrical field stimulation of corpus cavernosum isolated from the rabbit. In an anesthetized dog model for the evaluation of erectile function, intravenous administration of FR226807 prolonged the time to return to 75% of maximal intracavernosal pressure after cessation of electrical stimulation of the pelvic nerve. In summary, FR226807 is a potent and highly selective phosphodiesterase type 5 inhibitor with an augmentative effect on penile erection and will be useful for the treatment of erectile dysfunction. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Phosphodiesterase type 5 inhibitor; Penile erection; FR226807

1. Introduction

Penile erection is due to relaxation of corpus cavernosum smooth muscle during sexual stimulation (Lerner et al., 1993). The relaxation is mainly mediated by nitric oxide (NO) released from nonadrenergic, noncholinergic (NANC) neurons and the endothelium (Burnett et al., 1992). NO increases intracellular cGMP levels of the cavernosal smooth muscle cell by activating soluble guanylate cyclase, which catalyzes the conversion of guanosine 5′-triphosphate to cGMP.

The balance between synthetic enzyme (guanylate cyclase) and hydrolytic enzyme (cGMP phosphodiesterase) regulates intracellular cGMP levels. Therefore, agents that inhibit cGMP phosphodiesterase increase the cGMP levels in the corpus cavernosum and thereby facilitate the erectile response. Three isoenzymes of cyclic nucleotide phosphodiesterase, namely, phosphodiesterase type 2, phosphodiesterase type 3 and phosphodiesterase type 5, have been identified in extracts of human penis (Robert et al., 1999); however, phosphodiesterase type 5 is the predominant isozyme responsible for the metabolism of cGMP in the corpus cavernosum.

Human phosphodiesterases are encoded by at least 15 genes and have been classified into 10 families according to their chromatographic properties, substrate specificity and susceptibility to inactivation by inhibitors (Beavo, 1995; Fisher et al., 1998a,b). Inhibition of phosphodiesterases results in tissue-specific physiological functions because the distribution of phosphodiesterases varies in each tissue. A compound with phosphodiesterase isozyme selectivity is required because insufficient selectivity for the target phosphodiesterase leads to undesirable effects.

Here, we report the development of a new phosphodiesterase type 5 inhibitor, FR226807. The chemical structure of FR226807, *N*-(3,4-dimethoxybenzyl)-

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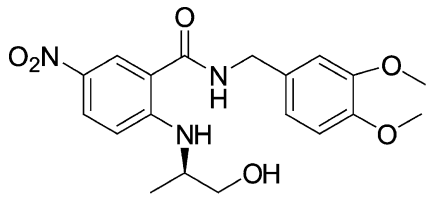

FR226807

Fig. 1. Structure of FR226807: *N*-(3,4-dimethoxybenzyl)-2-{[(1*R*)-2-hydroxy-1-methylethyl]amino}-5-nitrobenzamide.

2-{[(1*R*)-2-hydroxy-1-methylethyl]-amino}-5-nitrobenzamide, is shown in Fig. 1.

The aim of the present work is to investigate the pharmacological profile of FR226807 by using in vitro and in vivo assays. For this purpose, we assessed its effect on human phosphodiesterase isoenzymes and on the accumulation of cGMP in corpus cavernosum as indices of the in vitro potency of FR226807. Pharmacological evaluation of its effect on corpus cavernosum was performed both in an isolated rabbit preparation and in anesthetized dogs.

2. Materials and methods

2.1. Phosphodiesterase assay

2.1.1. Preparation of phosphodiesterase soluble fractions from tissues

All procedures were performed at 4 °C. Phosphodiesterase type 1 was isolated from umbilical arteries of human umbilical cords, which were a generous gift from Tukuba University, Japan. Umbilical arteries were dissected from umbilical cords and homogenized in 10 mM Tris/HCl homogenization buffer containing 250 mM sucrose, 1 mM EDTA, 2 mM benzamidine, and 0.2 mM phenylmethylsulfonyl fluoride, using a motor-driven Teflon-glass homogenizer, at pH 7.4. The homogenate was centrifuged at $100{,}000 \times g$ for 60 min. The soluble fraction was collected and used for the preparation of phosphodiesterase type 1. Phosphodiesterase types 2, 3 and 5 were isolated from human platelets. Platelets were obtained by centrifuging fresh human blood from healthy volunteers at $100 \times g$ for 15 min and then at $800 \times g$ for 15 min. Platelets were resuspended in ice-cold 20 mM Tris/HCl homogenization buffer containing 5 mM EDTA, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine, 2.5 IU/l aprotinin, and 20 mM magnesium acetate, at pH 7.5, and then homogenized using a motor-driven Teflon-glass homogenizer. The homogenate was centrifuged at $100{,}000 \times g$ for 60 min. The soluble fraction was collected and used for the preparation of phosphodiesterase types 2, 3 and 5. Phosphodiesterase type 4 was isolated from U937 human histiocytic lymphoma cell line in which phosphodiesterase type 4 have been identified rich (MacKenzie and Houslay, 2000; DiSanto and Heaslip, 1993). Cells were homogenized with ice-cold 20 mM Tris/HCl homogenization buffer containing 0.03% deoxycholic acid, 2 mM 2-mercaptoethanol, 1 μM leupeptin, 0.34 μM pepstatin A, 20 μM *N-p*-tosyl-l-lysine chloromethyl ketone, and 100 μM phenylmethylsulfonyl fluoride, using a motor-driven Teflon-glass homogenizer, at pH 8.0 and then centrifuged at $100{,}000 \times g$ for 60 min. The soluble fraction was collected and used directly for the phosphodiesterase activity assay. Phosphodiesterase type 6 was isolated from human retina. Human eyes were obtained from the Illinois eye-bank. Retinas were dissected free from eyes and homogenized in 20 mM 3-[*N*-morpholino] propanesulfonic acid (MOPS) buffer containing 47.5% sucrose, 2 mM $MgCl_2$, 60 mM KCl, 30 mM NaCl, 1 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride, using a motor-driven Teflon-glass homogenizer, at pH 7.2. The homogenate was centrifuged at $4000 \times g$ for 120 min. The supernatant was diluted with an equal volume of 20 mM MOPS buffer without sucrose and centrifuged at $16{,}000 \times g$ for 60 min. The pellet from the first centrifugation was extracted with 5 mM Tris/HCl containing 0.5mM EDTA, 1 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride, at pH 7.5. After centrifugation at $100{,}000 \times g$ for 45 min, the supernatant was added to the supernatant from the second centrifugation. The soluble fraction was collected and used for the preparation of phosphodiesterase type 6.

The soluble fraction was applied to a diethylaminoethyl-Sepharose column (Amersham Pharmacia Biotech) pre-equilibrated with 10 mM Tris/HCl buffer containing 70 mM sodium acetate, 2 mM magnesium acetate, 1 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride, at pH 6.5. The loaded column was first washed with 1000 ml of the 10 mM Tris/HCl buffer and then eluted using a continuous gradient of 70 to 1000 mM sodium acetate in 10 mM Tris/HCl buffer. The flow rate was 2.5 ml/min, and 5.2-ml fractions were collected. For phosphodiesterase type 6, the column was pre-equilibrated with 10 mM Tris/HCl buffer containing 1 mM $MgCl_2$, 1 mM dithiothreitol and 0.2 mM phenylmethylsulfonyl fluoride, at pH 7.5. The loaded column was first washed with 1000 ml of the 10 mM Tris/HCl buffer and then eluted using a continuous gradient of 30 to 400 mM NaCl in 10 mM Tris/HCl buffer. The flow rate was 2.5 ml/min, and 6-ml fractions were collected. Phosphodiesterase activity in the column fractions was determined as described below.

2.1.2. Phosphodiesterase assays for Sepharose chromatography fractions

Aliquots of the eluted fraction were incubated with 50 mM Tris/HCl buffer containing 0.1 μM [^{3}H]cGMP or 0.1 μM [^{3}H]cAMP, 10 U/ml crotalus atrox snake venom, 30 mM $MgCl_2$ and 0.1 mM dithiothreitol, 2 mM EGTA, at pH 7.5, for all phosphodiesterases except phosphodi-

esterase types 4 and 6. The snake venom was used for conversion of the 5′-mononucleotide to the nucleoside plus inorganic phosphate. [^{3}H]cGMP was used as the substrate for phosphodiesterase types 1, 5 and 6. [^{3}H]cAMP was used as the substrate for phosphodiesterase types 2, 3 and 4. 4 mM $CaCl_2$/10 U/ml calmodulin was added to target phosphodiesterase type 1, 10 μM cGMP to target phosphodiesterase type 2, 10 μM rolipram to target phosphodiesterase type 3, and 2 mM EGTA to target phosphodiesterase type 5. For phosphodiesterase type 6, 40 mM Tris/HCl buffer containing 0.1 μM [^{3}H]cGMP, 10 U /ml crotalus atrox snake venom, 10 mM $MgCl_2$ and 0.1 mM dithiothreitol, at pH 7.5, was used. Phosphodiesterase type 6 activity was activated by adding 50 μg/ml trypsin. The reaction was initiated by the addition of the radiolabeled substrate and incubated in a water bath at 30 °C for 10 min. The reaction was then stopped by addition of an anion exchanger. The reaction mixture was vortex-mixed and centrifuged at $800 \times g$ for 10 min. The resulting supernatant was transferred to Lumaplate®, a solid scintillator-coated 96-well microplate, and the radioactivity was measured by using a Topcounter®.

2.1.3. Inhibitory effect of FR226807 and sildenafil

Phosphodiesterase activity was measured in duplicate. For studies of the inhibition of the activity of phosphodiesterase isozymes, several concentrations of FR226807 or sildenafil were added to incubation mixtures. IC_{50} values were based on a fit to a linear regression logistic model.

2.2. Organ chamber experiment

2.2.1. Tissue preparation

The corpus cavernosum was excised from male Japan white rabbits (weighing 2.0–3.0 kg, from Kitayamarabesu, Japan) under anesthesia with sodium pentobarbital, and immediately placed in Krebs solution with the following composition: NaCl, 118 mM; $NaHCO_3$, 25 mM; KCl, 4.7 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; glucose, 10 mM; $CaCl_2$, 2.5 mM. Two strips of corpus cavernosum smooth muscle were dissected from one penis. Each strip was mounted between two metal hooks in an organ bath chamber containing 25 ml of Krebs solution. The tissue bath solution was maintained at 37 °C and aerated with a 95% O_2 and 5% CO_2 gas mixture. The tissue was stretched to a resting force of 0.25–0.4 g, and the isometric tension was recorded via a force-displacement transducer on an ink writing recorder (Nihon Kohden, Japan), and was equilibrated for at least 60 min. During this period, the tissue was washed with fresh solution every 15 min, and tension was adjusted if necessary.

2.2.2. Isometric tension and cGMP levels in corpus cavernosum

The strips of smooth muscle were contracted with norepinephrine (1 μM). After the contractile responses to norepinephrine had stabilized, FR226807 was added to the organ bath. When the relaxation response to FR226807 had reached its maximum, the tissue was immediately frozen in liquid nitrogen and stored at −80 °C. Homogenates were prepared in 1 ml of ice-cold 20 mM HEPES (pH 7.2) buffer containing 0.32 M sucrose, 0.5 mM EDTA, 1 mM dithiothreitol and protease inhibitors (3 μM leupeptin, 1 μM pepstatin A, and 1 mM phenylmethylsulfonyl fluoride), using a Polytron homogenizer. Cytosol and particulate fractions were separated by centrifugation at $12500 \times g$ for 60 min, at 4 °C. The cytosol fraction was acetylated, and the cGMP content was measured using a cyclic GMP [^{125}I] assay system (Amersham International, IL, USA). The sensitivity of this assay was 0.5 fmol. The cGMP content was corrected for the protein content. Protein concentrations in the cytosol were assayed with Bio-Rad protein assay reagent, using bovine serum albumin as a standard.

2.2.3. Electrical field stimulation-induced relaxation

Guanethidine (5 μM) and atropine (1 μM), to produce adrenergic and cholinergic blockade, respectively, were routinely added to the bath in the last 30 min of equilibration in order to detect the relaxation response to the stimulation of nonadrenergic, noncholinergic nerves. After an equilibration period, strips were contracted with 1 μM norepinephrine. After the norepinephrine contractile responses had stabilized, the tissue was subjected to the first electrical field stimulation-induced relaxation at 10 V (0.5-ms pulse duration), using sequential frequencies of 1, 3, 5, 15, 30 Hz delivered as 10-s trains. Thirty minutes after addition of FR226807 to the bath, the second electrical field stimulation was performed. The relaxation of corpus cavernosum to electrical field stimulation is expressed as the percent change in tone just before each stimulus and the duration is expressed as the time it took tension to return to the baseline just before each stimulus.

2.3. Measurement of intracavernosal pressure in dog

Male Beagle dogs (8.5–11.5 kg) were anesthetized with sodium pentobarbital, 25 mg/kg, administered intravenously via the brachial vein. Ten minutes later, sodium pentobarbital (2.5 mg/kg) was administered via the brachial vein. The right femoral vein and artery were cannulated for the administration of compounds and for the measurement of blood pressure, respectively. The cavernosal branch of the pelvic nerve was identified, dissected free from the surrounding tissue, and bipolar stimulating electrodes were placed on it. The penis was carefully denuded of skin down to the base and exposed. A 21-gauge scalp needle attached by a flexible catheter to a pressure transducer was inserted into the corpus cavernosum for the measurement of intracavernosal pressure. In our preliminary study, the pelvic nerve was stimulated at 10 V over the frequency of 3–10 Hz with a 0.2-ms pulse width for 1

min. The parameters which produced a submaximal response of intracavernosal pressure (10 V: 7 Hz with a 0.2-ms pulse width) were selected to evaluate the effect of FR226807 or sildenafil on intracavernosal pressure. The time it took for intracavernosal pressure to return at 75% of maximal intracavernosal pressure after cessation of stimulation was chosen as a pharmacological parameter of efficacy. Stimulation was repeated at least three times to obtain stable responses of intracavernosal pressure to electrical stimulation before administration of a test compound.

2.4. Materials

FR226807, sildenafil and rolipram were synthesized in Fujisawa Pharmaceutical (Osaka, Japan). Cyclic [^{3}H]AMP and cyclic [^{3}H]GMP was purchased from NEN Life Science. Guanethidine, atropine, EGTA, dithiothreitol, phenylmethylsulfonyl fluoride, calmodulin, and snake venom were purchased from Sigma. Norepinephrine was purchased from Sankyo. All other chemicals were of the purest commercially available grade.

2.5. Statistics

All data are expressed as means ± S.E.M. Student's *t*-tests were used to determine the significance of differences for means. $P < 0.05$ was considered statistically significant. For simultaneous multiple comparisons, the statistical significance of differences between groups was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. IC_{50} values were obtained by using non-linear curve fitting methods.

3. Results

3.1. Effect of FR226807 on phosphodiesterase isozymes

Inhibition profiles of FR226807 for phosphodiesterase isoenzymes are shown in Table 1. FR226807 potently inhibited phosphodiesterase type 5 with an IC_{50} value of 1.1 nM, which was fourfold lower than that of sildenafil (IC_{50} for phosphodiesterase type 5 was 4.3 nM). FR226807 also inhibited phosphodiesterase type 6 isolated from human retina with an IC_{50} value of 20 nM, but this IC_{50} value was about 18-fold higher than that for phosphodiesterase type 5. FR226807 showed very weak inhibitory activity for phosphodiesterase types 1, 2, 3 and 4. Sildenafil showed weak inhibitory activity for phosphodiesterase types 1, 2, 3 and 4; however, the IC_{50} value for phosphodiesterase type 6 was only 3.5-fold higher than that for phosphodiesterase type 5.

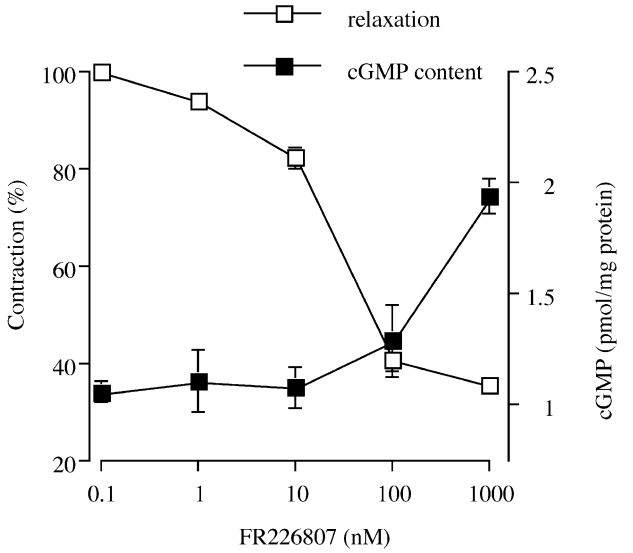

Fig. 2. Effect of FR226807 on contraction and cGMP content of rabbit corpus cavernosum ($N = 4$).

3.2. Effect of FR226807 on isometric tension and cGMP levels in corpus cavernosum

FR226807 caused a concentration-dependent relaxation in the isolated rabbit corpus cavernosum precontracted with norepinephrine. As shown Fig. 2, FR226807 dose-dependently increased cGMP levels from 1.06 to 1.94 pmol/mg protein. Relaxation elicited by FR226807 was associated with a rise in the tissue content of the cGMP.

Table 1
IC_{50} values of FR226807 and sildenafil on human phosphodiesterase isozymes

	IC50 (nM) ($n = 3$)					
	PDE 1	PDE2	PDE3	PDE4	PDE5	PDE6
FR226807	71 000	11 000	5400	8600	1.1	20
	40 000–170 000	5200–26 000	1400–14 000	5200–15 000	0.42–2.8	15–27
Sildenafil	1000	> 100 000	> 100 000	95 000	4.3	15
	370–2800	–	–	42 000–520 000	2.0–8.7	8.8–25

IC_{50} with 95% confidence intervals.
PDE 1 was isolated from human umbilical artery.
PDE 2, PDE 3 and PDE5 were isolated from human platelets.
PDE 4 was isolated from U937 cells (histiocytic lymphoma cell line).
PDE 6 was isolated from human retina.

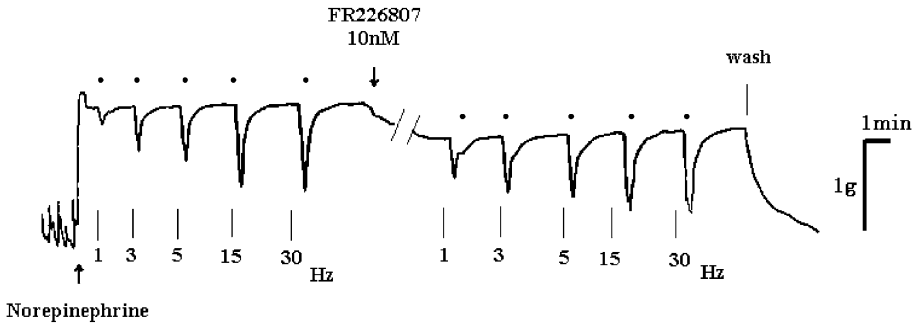

Fig. 3. Typical tracing showing the effect of FR226807 (10 nM) on the relaxation response induced by electrical field stimulation in rabbit corpus cavernosum precontracted with norepinephrine.

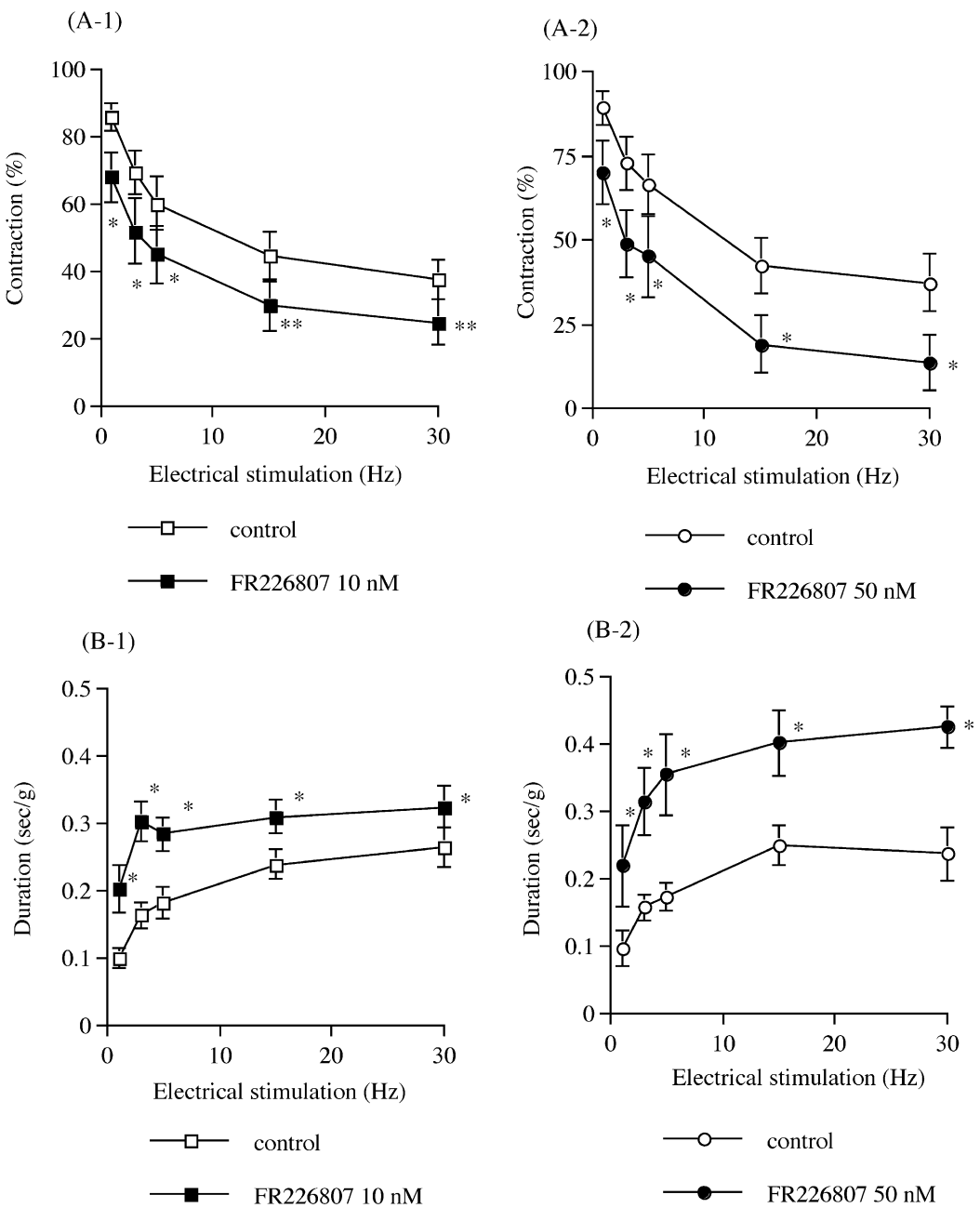

Fig. 4. Effect of FR226807 on the electrical field stimulation-induced relaxation (A) and duration of the contraction (B) of rabbit corpus cavernosum ($N = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus control.

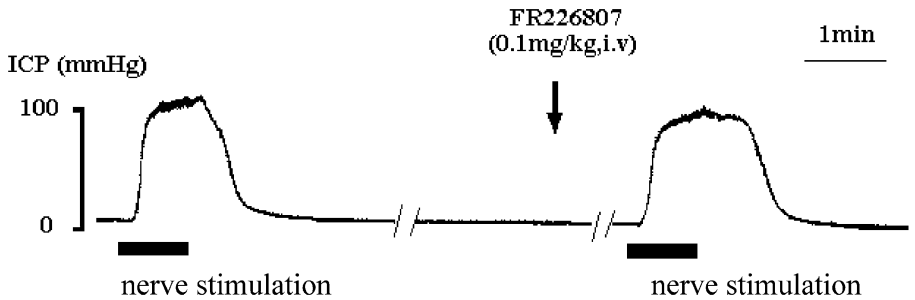

Fig. 5. Typical tracing showing the effects of FR226807 (0.1 mg/kg) on intracavernosal pressure in anesthetized dogs.

3.3. Effect of FR226807 on electrical field stimulation-induced relaxation of rabbit corpus cavernosum

A typical tracing is shown in Fig. 3. Electrical field stimulation caused frequency-dependent relaxation. Pretreatment with FR226807 potentiated the electrical field stimulation-induced relaxation in a concentration-dependent manner (Fig. 4). The most marked effect was observed at lower frequencies (225% at 1 Hz, 155% at 3 Hz, 139% at 5 Hz, 127% at 15 Hz, and 121% at 30 Hz at 10 nM FR226807). In addition to enhancing the magnitude of relaxation, FR226807 prolonged the duration of the relaxation response in a concentration-dependent manner, i.e. prolonged the time to return to baseline. The effect of FR226807 at 10 and 50 nM was statistically significant (Fig. 4).

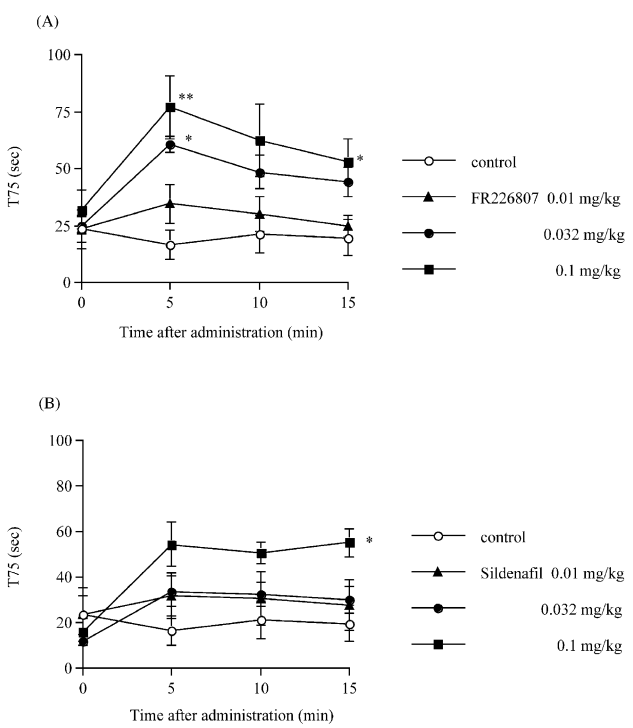

Fig. 6. Effect of FR226807 (A) and sildenafil (B) on the T75 of intracavernosal pressure in anesthetized dogs ($N = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus control.

3.4. Measurement of intracavernosal pressure and mean arterial pressure in dogs

In order to confirm effectiveness in vivo, the effect on intracavernosal pressure was evaluated in anesthetized dogs. A typical tracing is shown in Fig. 5. Electrical stimulation of the pelvic nerve induced a rapid increased in intracavernosal pressure, a test compound on the time to return to 75% of maximal intracavernosal pressure after the cessation of stimulation (T75) was increased at 5, 10 and 15 min after intravenous administration of a test compound. T75 was significantly prolonged by FR226807 at the doses of 0.032 and 0.1 mg/kg. The maximum effect of each dose was observed at 5 min, and this effect decreased in a time-dependent manner (Fig. 6). Sildenafil also prolonged T75; however, the effect was weaker than that of FR226807.

FR226807 induced a slight decrease in mean arterial pressure (control; $-2.4 \pm 0.92\%$, 0.01 mg/kg; $-7.7 \pm 2.4\%$, 0.032 mg/kg; $-9.2 \pm 1.4\%$, 0.1 mg/kg; $-8.55 \pm 1.2\%$) (Fig. 7). At the dose of 0.032 and 0.1 mg/kg, the change in mean arterial pressure was significantly different from that in the control ($P < 0.05$). In contrast, sildenafil caused a potent concentration-dependent decrease in mean arterial pressure (0.01 mg/kg; $-10.6 \pm 0.4\%$, 0.032 mg/kg; $-13.4 \pm 1.0\%$ and 0.1 mg/kg; $-20.4 \pm 4.0\%$),

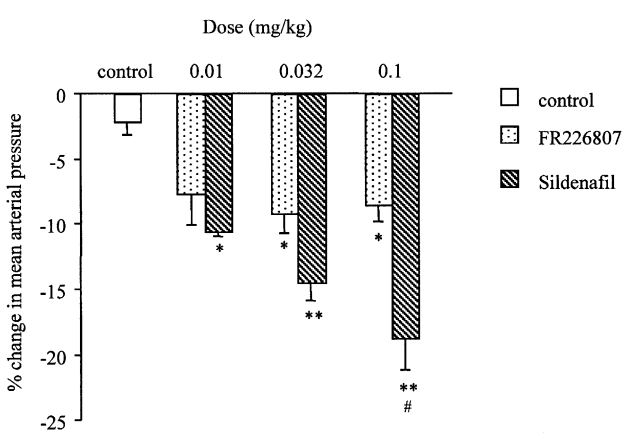

Fig. 7. Effect of FR226807 and sildenafil on mean arterial pressure in anesthetized dogs ($N = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus control, $^{\#}P < 0.05$ versus FR226807.

and these changes were statistically significant at all doses. At the dose of 0.032 and 0.1 mg/kg, there were significant differences in the decrease in mean arterial pressure between FR226807 and sildenafil ($P < 0.05$).

4. Discussion

FR226807 was discovered by screening for a compound with a high potency/high selectivity for phosphodiesterase type 5. This paper summarizes the pharmacological profile of FR226807 as a candidate for treatment of erectile dysfunction.

The characterization study for determining inhibitory activity against phosphodiesterases proved FR226807 to be a potent and selective inhibitor of phosphodiesterase type 5. FR226807 showed a high selectivity for phosphodiesterase type 5 relative to the other phosphodiesterases. For example, the inhibitory activity of FR226807 for phosphodiesterase type 5 was 18-fold more potent than that for phosphodiesterase type 6. Recently, sildenafil, a new phosphodiesterase type 5 inhibitor, was identified (Boolell et al., 1996). Sildenafil is the first oral medication approved for the treatment of erectile dysfunction. However, sildenafil is not yet an ideal compound because it causes side effects such as headache, flushing, dyspepsia, rhinitis and visual disturbances (Goldstein et al., 1998; Mark et al., 1999), possibly due to its insufficient selectivity for phosphodiesterase type 5. The first four side effects are speculated to be mainly caused by inhibition of phosphodiesterase type 5. The abnormal vision is most likely due to the weak interaction of sildenafil with phosphodiesterase type 6, the central effector enzyme involved in the pathway of visual excitation in photoreceptors (Helmreich and Hofmann, 1996). FR226807 is a more potent and a more specific phosphodiesterase type 5 inhibitor than sildenafil, so a lower incidence of visual disturbances would be anticipated in clinical use.

The primary mechanism for relaxation of corpus cavernosum smooth muscle and penile erection depends upon the NO-induced elevation of cGMP levels. It has been reported that the main phosphodiesterase that hydrolyzes cGMP in the corpus cavernosum is the phosphodiesterase type 5 and that a minor isozyme is phosphodiesterase type 2; phosphodiesterase type 1 has not been detected (Wallis et al., 1999). This means that agents such as phosphodiesterase type 5 inhibitors that enhance the NO–cGMP signal transduction pathway may prove beneficial in treating erectile dysfunction. In order to examine whether the FR226807-induced relaxation response is associated with the NO–cGMP pathway, we measured cGMP levels in the corpus cavernosum. FR226807 dose-dependently increased cGMP levels in the corpus cavernosum, an effect which was associated with relaxation of the corpus cavernosum. In addition, FR226807 potentiated the relaxation induced by sodium nitroprusside (data not shown). These results suggest that FR226807 elicited the response through the cGMP phosphodiesterase pathway.

It is now widely accepted that the relaxation response of corpus cavernosum is mainly mediated by NO released from NANC nerves. Electrical stimulation triggers an exclusively neurogenic response (Ignarro et al., 1990), that is, electrical field stimulation of isolated tissue is the most representative and suitable method for evaluating agents that modulate the NO–cGMP pathway. In our previous study, treatment with N^G-nitro-L-arginine methyl ester inhibited the electrical field stimulation-induced relaxation and this inhibition was reversed by addition of L-arginine, suggesting that the electrical field stimulation-induced relaxation was associated with the NO pathway. FR226807 enhanced the magnitude of the relaxation response of isolated corpus cavernosum and prolonged the duration of the relaxation response in a concentration-dependent manner, which indicated that FR226807 is expected to enhance penile erection in vivo.

Penile erection has been studied in vivo using rabbits, dogs and monkeys (Azadzoi and De Tejeda, 1991; Trigo-Rocha et al., 1993, 1994). However, the dog is considered to be the “best” species because various hemodynamic variables, such as blood pressure, heart rate, regional blood flow, and vascular resistance, can be monitored simultaneously. In general, the modulation of the increase in intracavernosal pressure induced by electrical stimulation of the cavernosal branch of the pelvic nerve is often used as model for the pharmacological evaluation of drugs. We preferred to measure the time to return to 75% of maximal intracavernosal pressure after cessation of electric stimulation rather than the intracavernosal pressure itself during electrical stimulation. FR226807 as well as sildenafil prolonged the T75 of intracavernosal pressure under our experimental conditions. We chose this method because the time to return to 75% of maximal intracavernosal pressure after cessation of electric stimulation is more sensitive to phosphodiesterase type 5 inhibitors and shows greater precision than does the magnitude of intracavernosal pressure. In addition, reported that a high concentration of NO in the corpus cavernosum alone was not sufficient to sustain the erectile response. This indicated that NO plays a very important role not only in erection but also in detumescence (Anderson, 1993). Delaying the decrease in intracavernosal pressure and increasing intracavernosal pressure are important aspects of the treatment of erectile dysfunction because most patients with erectile dysfunction have an inability to retain blood within the sinusoids of the penis, so-called venous leakage (Fuchs et al., 1989).

FR226807 had less effect than sildenafil on blood pressure at all doses, suggesting that FR226807 may have fewer side effects caused by vasodilation. One possibility is that FR226807 has less effect on phosphodiesterase type 1 than does sildenafil, because it has been reported that phosphodiesterase type 1 as well as phosphodiesterase type

5 participate in the regulation of blood pressure (Vemulapalli et al., 1996). However, this is an insufficient explanation for this smaller effect of FR226807 on blood pressure.

In conclusion, the present study showed that FR226807 is a potent and specific inhibitor of phosphodiesterase type 5, and a higher efficacy and a lower incidence of side-effects will be anticipated in the clinical use of FR226807 for the treatment of erectile dysfunction.

References

Anderson, K.E., 1993. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol. Rev. 45, 253–308.

Azadzoi, K.M., De Tejeda, I.S., 1991. Hypercholesterolemia impairs endothelium dependent relaxation of rabbit corpus cavernosum smooth muscle. J. Urol. 146, 238–240.

Beavo, J.A., 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev. 75, 725–748.

Boolell, M., Allen, M.J., Ballard, S.A., Gepi-Attee, S., Muirhead, G.J., Naylor, A.M., Osterloh, I.H., Gingell, C., 1996. Sildenafil: an oral active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. Int. J. Impo. Res. 8, 47–52.

Burnett, A.L., Lowenstein, C.J., Bredt, D.S., Chang, T.S.K., Snyder, S.H., 1992. Nitric oxide: a physiologic mediator of penile erection. Science 257, 401–403.

DiSanto, M.E., Heaslip, R.J., 1993. Identification and stabilization of large molecular weight PDE-IVs from U937 cells. Biochem. Biophys. Res. Commun. 197, 1126–1131.

Fisher, A.D., Smith, J.F., Pillar, J.S., St.Denis, S.H., Cheng, J.B., 1998a. Isolation and characterization of phosphodiesterase8A, a novel human cAMP-specific phosphodiesterase. Biochem. Biophys. Res. Commun. 246, 570–577.

Fisher, A.D., Smith, J.F., Pillar, J.S., St.Denis, S.H., Cheng, J.B., 1998b. Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. Biochem. Biophys. Res. Commun. 273, 15559–15564.

Fuchs, A.M., Mehringer, C.M., Rajfer, J., 1989. Anatomy of penile venous drainage in potent and impotent men during cavernosography. J. Urol. 141, 1353–1356.

Goldstein, I., Lue, T.F., Padma-Nathan, H., Rosen, R.C., Steers, W.D., Wicker, P.A., 1998. Oral Sildenafil in the treatment of erectile dysfunction. Sildenafil Study Group. N. Engl. J. Med. 338, 1397–1404.

Helmreich, E.J.M., Hofmann, K.P., 1996. Structure and function of proteins in G-protein-coupled signal transfer. Biochim. Biophys. Acta 1286, 285–322.

Ignarro, L.J., Bush, P.A., Buga, G.M., Wood, K.S., Fukuto, J.M., Rajfer, J., 1990. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem. Biophys. Res. Commun. 170, 843–850.

Lerner, S.E., Melman, A., Christ, G.J., 1993. A review of erectile dysfunction: new insights and more questions. J. Urol. 149, 1246–1255.

MacKenzie, S.J., Houslay, M.D., 2000. Action of rolipram on specific PDE4 cAMP phosphodiesterase isoforms and on the phosphorylation of cAMP-response-element-binding protein (CREB) and p38 mitogen-activated protein (MAP) kinase in U937 monocytic cells. Biochem. J. 347, 571–578.

Mark, B.N., George, J.C., Arnold, M., 1999. Sildenafil: a new oral therapy for erectile dysfunction. Drugs Today 35, 211–217.

Robert, M.W., Jackie, D.C., Sharron, H.F., Peter, E., 1999. Tissue distribution of phosphodiesterase families and the effect of Sildenafil on tissue cyclic nucleotide, platelet function, and the contractile responses of trabeculae carneae and aortic rings in vitro. Am. J. Cardiol. 83, 3C–12C.

Trigo-Rocha, F., Aronson, W.J., Hohenfellner, M., Ignarro, L.J., Rajfer, J., Lue, T.F., 1993. Nitric oxide and cGMP: mediators of pelvic nerve-stimulated erection in dogs. Am. J. Physiol. 264, H419–H422.

Trigo-Rocha, F., Hsu, G.L., Donatucci, C.F., Martinez-Pineiro, L., Lue, T.F., Tanagho, E.A., 1994. Intracellular mechanism of penile erection in monkeys. Neurourol. Urodyn. 13, 71–80.

Vemulapalli, S., Watkins, R.W., Chintala, M., Davis, H., Ahn, H.S., Fawzi, A., Tulshian, D., Chiu, P., Chatterjee, M., Lin, C.C., Sybertz, E.J., 1996. Antiplatelet and antiproliferative effects of SCH 51866, a novel type 1 and type 5 phosphodiesterase inhibitor. J. Cardiovasc. Pharmacol. 28, 862–869.

Wallis, R.M., Corbin, J.D., Francis, S.H., Ellis, P., 1999. Tissue distribution of phosphodiesterase families and the effects of sildenafil on tissue cyclic nucleotides, platelet function, and the contractile responses of trabeculae carneae and aortic rings in vitro. Am. J. Cardiol. 4, 3C–12C.