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# T-0156, a novel phosphodiesterase type 5 inhibitor, and sildenafil have different pharmacological effects on penile tumescence and electroretinogram in dogs

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**Abstract**

T-0156 (2-(2-methylpyridin-4-yl)methyl-4-(3,4,5-trimethoxyphenyl)-8-(pyrimidin-2-yl)methoxy-1,2-dihydro-1-oxo-2,7-naphthyridine-3-carboxylic acid methyl ester hydrochloride) is a newly synthesized phosphodiesterase type 5 inhibitor, and its potency and selectivity are higher than those of sildenafil in an enzyme assay. In the present study with anesthetized dogs, we examined the effects of intravenous T-0156 or sildenafil on the pelvic nerve stimulation-induced penile tumescence and light-adapted flicker stimulation-induced electroretinogram, parameters of which are reported to be indicators for inhibition of phosphodiesterase type 5 and type 6, respectively. Both compounds potentiated the penile tumescence in a dose-dependent manner. T-0156 at 10 μg/kg and sildenafil at 100 μg/kg showed almost the same potentiating percentage (181.5 ± 31.1% and 190.0 ± 37.9%) in spite of the plasma concentration of T-0156 being about five times lower than that of sildenafil (16.7 ± 1.6 and 78.8 ± 5.3 ng/ml), indicating that the effect of T-0156 on tumescence is more potent than that of sildenafil. While the high dose of T-0156 (1000 μg/kg) reduced the amplitude and increased the latency of the electroretinogram positive wave, the effects of T-0156 were conversely weaker than those of sildenafil (reduction of amplitude; T-0156: 41.1 ± 8.0%, sildenafil: 71.7 ± 3.9%, increase of latency; T-0156: 3.9 ± 0.6%, sildenafil: 14.5 ± 1.4%, at 1000 μg/kg). These results clearly showed the difference in the properties of T-0156 and sildenafil in pharmacological studies with anesthetized dogs, and the difference appeared to correspond with their inhibitory potencies for phosphodiesterase type 5 and type 6. It was concluded that T-0156 would be a useful pharmacological tool as a potent and highly selective phosphodiesterase type 5 inhibitor.
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*Keywords:* T-0156; Sildenafil; Phosphodiesterase type 5 inhibitor; Phosphodiesterase type 6; Penile tumescence; Electroretinogram

## 1. Introduction

Cyclic guanosine monophosphate (cGMP) is an intracellular second messenger and plays an important role in the regulation of many physiological functions such as smooth muscle relaxation and visual phototransduction (Waldman and Murad, 1987; Furchgott and Vanhoutte, 1989). Intracellular cGMP levels are regulated by the balance between the synthesis by guanylate cyclase and the hydrolysis by phosphodiesterase. There are at least 11 distinct phosphodiesterase isozymes (types 1–11), and type 5, type 6, and type 9 are cGMP-specific phosphodiesterases (Beavo, 1995; Soderling et al., 1998a,b; Soderling et al., 1999; Fawcett et al., 2000). Therefore, the inhibition of these cGMP-specific phosphodiesterases is assumed to be crucial for the change in intracellular cGMP levels and cGMP-dependent physiological responses. Recently, this hypothesis has been supported by accumulating evidence from work with phosphodiesterase type 5 inhibitors (Beavo, 1995; Noto et al., 2000; Inoue et al., 2001; Takagi et al., 2001).

Sildenafil is a typical phosphodiesterase type 5 inhibitor ($IC_{50}$ value of 3.5 nM; Ballard et al., 1998) and has been used for the evaluation of physiological roles of phosphodiesterase type 5 in pharmacological experiments. In accordance with the hypothesis, sildenafil potentiates cGMP-dependent relaxation in the isolated corpus cavernosum (Chuang et al., 1998; Takagi et al., 2001) and facilitates pelvic nerve stimulation-induced penile tumescence mediated by a nitric oxide (NO)/cGMP system (Carter et al.,

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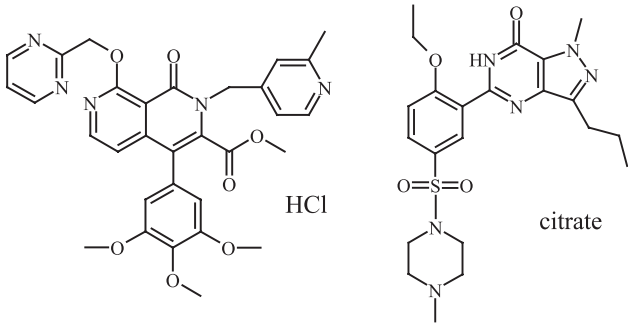


Fig. 1. Chemical structure of T-0156 and sildenafil.

1998; Noto et al., 2000; Gemalmaz et al., 2001). These effects of sildenafil are based on the fact that phosphodiesterase type 5 is a predominant cGMP-hydrolyzing enzyme in the corpus cavernosum (Boolell et al., 1996). On the other hand, sildenafil also inhibits phosphodiesterase type 1 and type 6 ($IC_{50}$ values of 281 and 33 nM, respectively; Ballard et al., 1998). Therefore, the inhibitory selectivity of sildenafil for phosphodiesterases is not very high.

T-0156, a naphthyridinone derivative, is a potent and highly selective phosphodiesterase type 5 inhibitor, and the selectivity of T-0156 for phosphodiesterase type 5 over type 1 and type 6 is greater than that of sildenafil (Ukita et al., 2003; Mochida et al., 2002, see Fig. 1 and Table 1). Therefore, T-0156 is expected to have pharmacological properties different from those of sildenafil on penile tumescence and on the electroretinogram, because these biological parameters are reported to be indicators for inhibition of phosphodiesterase type 5 and type 6, respectively (Noto et al., 2000; Estrade et al., 1998). In order to confirm this expectation, we examined the effects of T-0156 and sildenafil on the pelvic nerve stimulation-induced penile tumescence and light-adapted flicker stimulation-induced electroretinogram in anesthetized dogs.

## 2. Materials and methods

This study was approved by the Animal Research Committee of Tanabe Seiyaku.

### 2.1. Penile tumescence induced by pelvic nerve stimulation

Dogs weighing 10 to 18 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v. bolus injection, followed by 4.5 mg/kg/h i.v. infusion). An endotracheal tube was placed for ventilation (15 ml/kg/stroke, 20 strokes/min) with room air. Polyethylene tubes were cannulated into femoral artery for measuring mean arterial pressure, and into the axillary artery for collection of blood. The left pelvic nerve, located superior and lateral to the prostate, was carefully isolated and placed on a bipolar electrode (IMT-1530; Inter Medical, Nagoya, Japan). A 21-gauge venous needle was placed in the corpus cavernosum on the left side and used for recording intracavernous pressure. The pelvic nerve was stimulated by electrical square pulses (10 V, 0.2-ms pulse duration, for 40 s) at frequencies from 2.5 to 20 Hz at intervals of 20 min. All experiments were started when the submaximal nerve stimulation evoked consistent responses. Vehicle (0.01 N HCl/saline) or test compounds (3, 10, 30 or 100 μg/kg, i.v.) were administered cumulatively 5 min before the nerve stimulation. For quantitative determination of the tumescence, we measured the area under the curve (AUC) expressed as millimeters of mercury multiplied by minutes, and the AUC value was divided by mean arterial pressure. The effects of test compounds were expressed as percent changes of the pretreatment value of the tumescence.

Blood samples were collected 3 min after intravenous administration of test compounds. The plasma fraction was separated by centrifugation (3500 rpm, 4 °C, 10 min) and stored at −20 °C until the determination of plasma concentrations of compounds by high performance liquid chromatography (HPLC).

### 2.2. Electroretinogram induced by a light-adapted flicker stimulation

Dogs weighing 10 to 18 kg were anesthetized and ventilated as described above. To avoid effects of electromusculogram, vecuronium bromide, a muscle relaxant, was administered intravenously (40 + 400 mg/kg/h). Mydriasis was achieved with topical 0.5% tropicamide and 0.5%

Table 1
Inhibitory effects of T-0156 and sildenafil on phosphodiesterase isozymes

| Compounds | $IC_{50}$ (nM) | | | | | |
|---|---|---|---|---|---|---|
| | PDE1 | PDE2 | PDE3 | PDE4 | PDE5 | PDE6 |
| T-0156 | >100,000 | >100,000 | >100,000 | 63,000 ± 31,000 | 0.23 ± 0.027 | 56 ± 1.9 |
| Sildenafil | 270 ± 38 | 43,000 ± 1500 | >100,000 | 11,000 ± 3100 | 3.6 ± 0.26 | 29 ± 0.93 |

Phosphodiesterase type 1, type 4, and type 5 were isolated from canine lung, type 3 was isolated from canine heart. Phosphodiesterase type 2 and type 6 were prepared from canine adrenal gland and canine retinas, respectively. Phosphodiesterase type 1, type 2, and type 5 activities were assayed with 1 μM cGMP as a substrate, and type 3 and type 4 activities were assayed with 1 μM cAMP. After phosphodiesterase type 6 was activated by trypsin, the activity was measured with 10 μM cGMP. In each experiment, phosphodiesterase activity was 2–35 pmol/min/ml. The $IC_{50}$ values were determined by nonlinear regression (sigmoidal curve fit). Data are shown as means ± S.E.M. for three to four experiments. The data were cited from previous studies (Mochida et al., 2002).

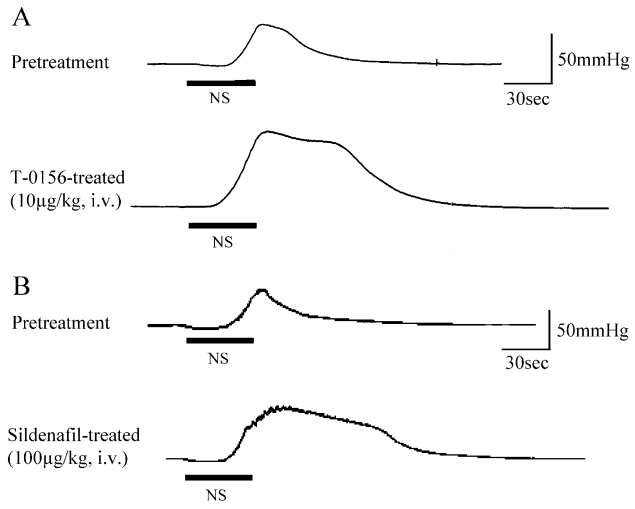


Fig. 2. Typical tracings showing the penile tumescence induced by pelvic nerve stimulation in the presence and absence of T-0156 or sildenafil in anesthetized dogs. T-0156 or sildenafil was administered intravenously 5 min before the stimulation. NS: nerve stimulation.

phenylephrine hydrochloride drops (Midrin-P). Contact lens electrode (type AII, 23 mm, Kyoto Contact Lens, Kyoto, Japan) and ground electrode were attached to the cornea and the lower lip. To protect the cornea, 1.5% hydroxyethylcellulose gel (Scopisol 15) was used on the inner contact lens surface. After light adaptation (42–48 cd/m$^2$), the retina was stimulated repeatedly at intervals of 20 min with a flicker light (59–64 cd s/m$^2$, 30 Hz, 200 times) using a Ganzfeld stimulator (2503S, LKC Technologies, Maryland, USA). The experiments were started after the waveform was stabilized. Vehicle (0.01 N HCl/saline) or test compounds (100, 300, 1000 or 3000 μg/kg, i.v.) were administered cumulatively 5 min before the retina stimulation. The electroretinogram induced by light stimulation was measured and averaged automatically using an evoked potential measuring system (MEB5504, Nihon Kohden, Tokyo, Japan). The peak response and implicit time of positive wave of the electroretinogram were detected as the amplitude and latency. The effects of test compounds were expressed as percent changes of the pretreatment value of the amplitude or latency.

### 2.3. Chemicals

T-0156 and sildenafil were provided by Sigma-Aldrich (MA, USA) and Medicinal Chemistry Research Laboratories, Tanabe Seiyaku (Saitama, Japan), respectively. Vecuronium bromide (Masculax Intravenous TD), Midrin-P, and Scopisol 15 were purchased from Sankyo (Tokyo, Japan), Santen Pharmaceutical (Osaka, Japan), and Senju Pharmaceutical (Osaka, Japan), respectively. T-0156 and sildenafil were dissolved in 0.01 N HCl/saline, and sodium pentobarbital and vecuronium bromide were dissolved in distilled water.

### 2.4. Data analysis

Statistical analysis was performed with a one-way analysis of variance (ANOVA) with randomized complete block design followed by Dunnett's test (versus vehicle) and Student's *t*-test (unpaired values, T-0156 vs. sildenafil) in Figs. 3 and 6. *P*-values $<0.05$ were considered as statistically significant. Data are shown as means ± S.E.M.

## 3. Results

### 3.1. Pelvic nerve stimulation-induced penile tumescence

Typical tracings (Fig. 2) show the effect of intravenous T-0156 and sildenafil on the penile tumescence induced by pelvic nerve stimulation in anesthetized dogs. Pelvic nerve stimulation for 40 s caused an increase in intracavernous pressure with a time lag of approximately 20 s. The elevated pressure returned to its basal level after the termination of nerve stimulation. T-0156 at 10 μg/kg enhanced the penile tumescence with a prolongation of duration (Fig. 2A). Sildenafil at 100 μg/kg also enhanced the penile tumescence to the same extent (Fig. 2B).

Fig. 3 shows dose–response curves for the potentiating effect of T-0156 and sildenafil on the penile tumescence induced by pelvic nerve stimulation in anesthetized dogs. Both compounds potentiated the penile tumescence in a dose-dependent manner and the potentiations at 10 to 100 μg/kg were statistically significant ($P<0.05$). The potentiating percentage by T-0156 at 10 μg/kg was significantly higher than that by the same dose of sildenafil (T-0156: 181.5 ± 31.1%, sildenafil: 64.4 ± 13.6%). Plasma levels of T-0156 were about twofold higher than those of sildenafil at every dose (example; T-0156: 16.7 ± 1.6 ng/ml, sildenafil: 7.9 ± 0.2 ng/ml, at 10 μg/kg; Fig. 4).

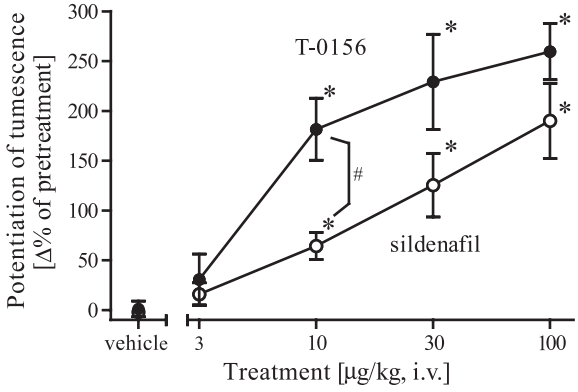


Fig. 3. Effect of intravenous treatment with T-0156 or sildenafil on the pelvic nerve stimulation-induced penile tumescence in anesthetized dogs. The effects of test compounds were expressed as percent change of the pretreatment value of the tumescence. Data are shown as means ± S.E.M. for four or five dogs. *$P<0.05$ vs. vehicle treatment. $^{\#}P<0.05$, T-0156 vs. sildenafil.

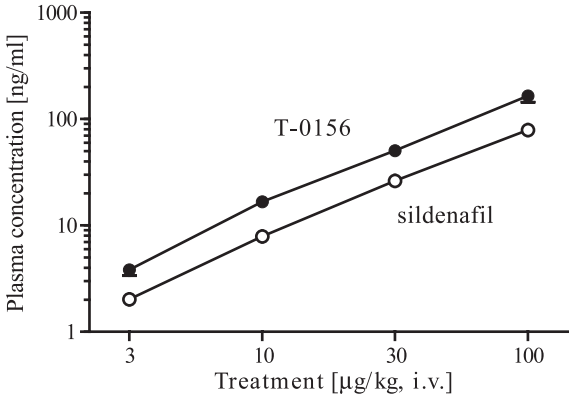


Fig. 4. Plasma levels of T-0156 and sildenafil in blood samples collected at 3 min after intravenous administration in anesthetized dogs. Data are shown as means ± S.E.M. for four or five dogs.

### 3.2. Light-adapted flicker stimulation-induced electroretinogram

Fig. 5 shows typical tracings for the effect of intravenous T-0156 and sildenafil on the electroretinogram induced by a light-adapted flicker stimulation in anesthetized dogs. The stimulation caused a positive wave of the electroretinogram. T-0156 at 100 and 300 μg/kg had no, or a slight, effect on the waveform. T-0156 at 1000 μg/kg reduced the amplitude and increased the latency of the positive wave (Fig. 5A). Sildenafil at 100 to 1000 μg/kg reduced the amplitude and increased the latency of the positive wave in a dose-dependent manner (Fig. 5B). Both compounds at 3000 μg/kg greatly flattened the waveform of electroretinogram, and the peak of positive wave was not quantified (data not shown).

Fig. 6 shows dose–response curves for the effects of T-0156 and sildenafil on the amplitude (Fig. 6A) and latency

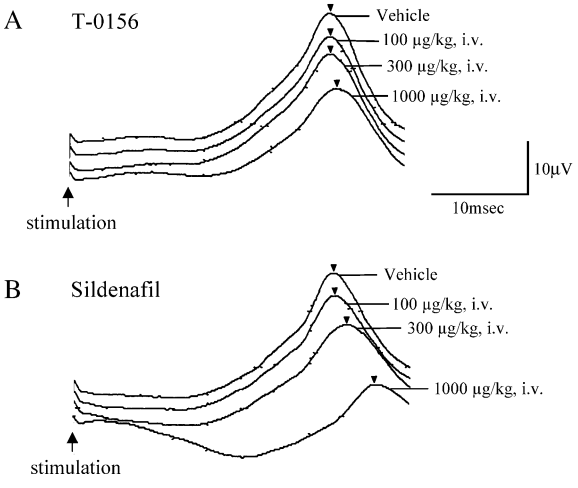


Fig. 5. Typical tracings showing the light-adapted electroretinogram induced by 30-Hz flicker stimulations in the presence and absence of T-0156 or sildenafil in anesthetized dogs. Each curve is the average of responses to the flicker stimulations (200 times). T-0156 or sildenafil was administered intravenously 5 min before the stimulation.

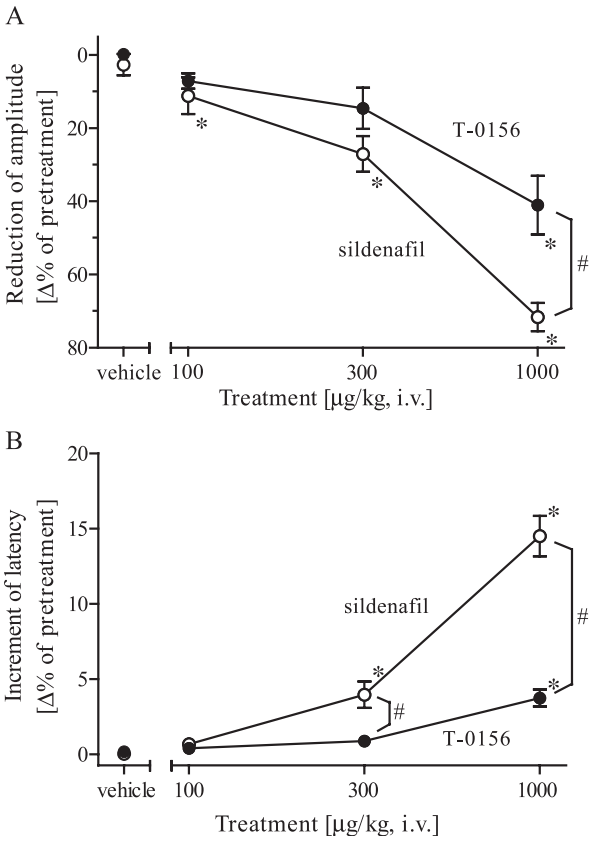


Fig. 6. Effect of intravenous treatment with T-0156 or sildenafil on the amplitude (A) and latency (B) of the positive wave of the electroretinogram induced by 30-Hz flicker stimulations in anesthetized dogs. The effects of test compounds were expressed as percent change of the pretreatment value of each parameter. Data are shown as means ± S.E.M. for four dogs. *$P<$ 0.05 vs. vehicle treatment. #$P<$ 0.05, T-0156 vs. sildenafil.

(Fig. 6B) of the positive wave of the electroretinogram induced by a light-adapted flicker stimulation. Regarding the effect on amplitude, both compounds produced a dose-dependent reduction, and the effect of T-0156 at 1000 μg/kg and that of sildenafil at 100 to 1000 μg/kg was statistically significant. The percentage of the reduction by sildenafil at 1000 μg/kg was significantly higher than that by the same dose of T-0156 (T-0156: 41.1 ± 8.0%, sildenafil: 71.7 ± 3.9%). For the effect on latency, T-0156 at 1000 μg/kg only produced the significant increase, while sildenafil at 300 to 1000 μg/kg produced the significant increase in a dose-dependent manner. The percentage of the increase by sildenafil at 300 to 1000 μg/kg was significantly higher than that by the same dose of T-0156 (T-0156: 3.9 ± 0.6%, sildenafil: 14.5 ± 1.4%, at 1000 μg/kg).

## 4. Discussion

The present study showed that T-0156, a novel phosphodiesterase type 5 inhibitor, had pharmacological properties different from those of sildenafil on the penile tumescence and electroretinogram in anesthetized dogs, and the differ-

ence most likely reflects the inhibitory activities for phosphodiesterase type 5 and type 6, as discussed below.

The pelvic nerve stimulation-induced tumescence has been used as a model for the pharmacological evaluation of phosphodiesterase type 5 inhibitors. It has been already reported that intraduodenal treatment with T-0156 or sildenafil induces a dose-dependent potentiation of tumescence in this model (Noto et al., 2000; Mochida et al., 2002). In the present study, we used this identical animal model in order to evaluate the effect of intravenous treatment with T-0156 or sildenafil. Both compounds potentiated the tumescence at the same doses, 10 μg/kg or more, and the potentiating percentage by T-0156 at 10 μg/kg was higher than that by sildenafil. This greater potentiating effect of T-0156 may reflect the plasma concentration of this compound, which was about twice that of sildenafil at the same dose. However, comparing T-0156 at 10 μg/kg with sildenafil at 100 μg/kg shows almost the same potentiating percentage (181.5 ± 31.1% and 190.0 ± 37.9%); the plasma concentration of T-0156 was about fivefold lower than that of sildenafil (16.7 ± 1.6 ng/ml and 78.8 ± 5.3 ng/ml), indicating that the potentiating effect of T-0156 on tumescence is more potent than that of sildenafil. In enzyme assays, T-0156 showed inhibition of phosphodiesterase type 5 with an $IC_{50}$ value of 0.23 nM, which is more potent than that of sildenafil ($IC_{50}$ value of 3.6 nM) (Mochida et al., 2002). It seems that the potentiating effect of these compounds on the tumescence reflects inhibitory activities for phosphodiesterase type 5. In our preliminary study, the maximum potentiating responses of tumescence with T-0156 and sildenafil were equal in this model (about 250%), and sildenafil at 300 μg/kg was necessary for the maximum response while T-0156 reached it at 100 μg/kg.

Cyclic GMP is also a key messenger involved in the phototransduction process of photoreceptor cells, rods and cones, of the retina (DeVries and Baylor, 1993; Yau, 1994). Light activation of the visual pigment is a first step in phototransduction causing the activation of phosphodiesterase type 6, which is present in high concentrations in photoreceptors (Beavo, 1995), via the activation of GTP-binding protein. The activation of phosphodiesterase type 6 decreases the cGMP levels, resulting in the closing of cGMP-dependent $Na^+$ channels and in the hyperpolarization of photoreceptors. The electrical impulses of photoreceptors evoked by light are transduced to the optic fiber through the cell layers of the retina, and these electrical transductions are reflected on the electroretinogram. In the present study, T-0156 and sildenafil showed effects on the waveform of the electroretinogram, indicating modification of photoreceptor function. The effects of sildenafil on the amplitude and latency of the positive wave of the electroretinogram were more potent than those of T-0156, despite the fact the plasma concentration of sildenafil was expected to be lower than that of T-0156 at the same doses. These results seem to reflect the potential relations in the enzyme assay in which the $IC_{50}$ value of sildenafil for phosphodiesterase type 6 (29 nM) was lower than that of T-0156 (56 nM). Furthermore, T-1032, which has almost the same $IC_{50}$ value for phosphodiesterase type 6 as sildenafil (28 nM; Kotera et al., 2000), also showed effects on the electroretinogram equal to those of sildenafil (reduction of amplitude: 66.2%, increment of latency: 9.7%, at 1000 μg/kg; our preliminary data), supporting the good correlation between the waveform alterations of the electroretinogram in vivo and the in vitro enzymatic inhibitory potency for phosphodiesterase type 6. Our results are consistent with the observations of Estrade et al. (1998) showing a correlation between the efficacy on the electroretinogram in isolated rat retina and the in vitro inhibitory potency for phosphodiesterase type 6.

The effects of sildenafil on the light-adapted flicker electroretinogram observed in the present study seems more potent than those on the dark-adapted electroretinogram in mice and dogs (Barry et al., 1998; Behn and Potter, 2001). These differences in the potency of sildenafil might be due to the procedure for retinal stimulation. We used the light-adapted flicker stimulation, which evoked the electroretinogram by cones contributing the sense of color (Schneider and Zrenner, 1986), because abnormal color vision has been reported by the people treated with sildenafil (Goldstein et al., 1998; Conti et al., 1999). These data indicate that the effect of sildenafil on the photopic vision is marked compared to that on the scotopic vision. Similar data have been reported for healthy volunteers given sildenafil orally (Luu et al., 2001).

We used the potentiation of the penile tumescence and the alteration of electroretinogram waveform as indicators for phosphodiesterase type 5 and type 6 inhibition, respectively. However, it seems difficult to show the inhibitory selectivity for phosphodiesterase type 5 over type 6 in vivo, because of the difference in parameters analyzed, area under the curve of intracavernous pressure and amplitude or latency of electroretinogram positive wave. Therefore, we compared the in vivo efficacy with the in vitro enzymatic inhibitory potency of T-0156 and sildenafil, which are the phosphodiesterase inhibitors showing a different inhibitory potency for each phosphodiesterase subtype. As a result, it seems to have been shown that the differences in the inhibitory potency of these compounds in vitro were reflected by the in vivo efficacy.

There is a possibility that the physical and pharmacokinetic properties of test compounds affect the results of in vivo testing. These properties concern the plasma concentration and decay time of the compound. In the present study, we measured the plasma levels of compounds 3 min after their intravenous administration in the experiments on penile tumescence. The plasma levels of T-0156 were about twofold higher than those of sildenafil, and the ratio of the plasma levels of T-0156 to those of sildenafil was the same at every dose. Under these conditions, T-0156 showed a more potent effect on penile tumescence and weaker effects on electroretinogram than those of sildenafil. Therefore, it seems that the differential potencies of these compounds

observed in the present study are associated with the difference in pharmacological properties, not with physical and pharmacokinetic ones.

In conclusion, the results of the present in vivo study clearly showed that T-0156 had pharmacological properties different from those of sildenafil on the penile tumescence and electroretinogram in dogs. These pharmacological differences most likely reflect the inhibitory potencies and selectivity for phosphodiesterase type 5 and type 6. Furthermore, it was proved that T-0156 is a potent and highly selective phosphodiesterase type 5 inhibitor compared with sildenafil. T-0156 is considered to be the better tool as a specific phosphodiesterase type 5 inhibitor for pharmacological studies.

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