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Pharmacological profile of T-1032, a novel specific phosphodiesterase type 5 inhibitor, in isolated rat aorta and rabbit corpus cavernosum

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Abstract

This study was designed to examine the pharmacological properties of T-1032 (methyl-2-(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinoline carboxylate sulfate), a novel phosphodiesterase type 5 inhibitor, in isolated rat aorta and rabbit corpus cavernosum. T-1032 (3×10^{-11} to 3×10^{-7} M) caused an endothelium-dependent relaxation in the isolated rat aorta precontracted with phenylephrine, and the relaxation was accompanied by an increase in cGMP but not cAMP levels. The T-1032-induced relaxation was attenuated by N^{G}-nitro-L-arginine methyl ester (L-NAME) (10^{-3} M), a nitric oxide (NO) synthase inhibitor, or 1*H*-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) (10^{-5} M), a guanylyl cyclase inhibitor. T-1032 (10^{-9}, 10^{-8} M) produced a potentiation of the relaxation induced by sodium nitroprusside, but not of the relaxation induced by isoproterenol. In the isolated rabbit corpus cavernosum precontracted with phenylephrine, the electrical field stimulation-induced relaxation was attenuated by treatment with tetrodotoxin (10^{-6} M) as well as L-NAME (10^{-4} M). The L-NAME-inhibited relaxation was restored by treatment with L-arginine (5×10^{-4} M). T-1032 (10^{-9} to 10^{-6} M) and sildenafil (10^{-9} to 10^{-6} M) produced a potentiation of the electrical field stimulation-induced relaxation as well as a decrease in basal tension in a concentration-dependent manner. It was concluded that T-1032 had potentiating effects on the NO/cGMP signaling pathway in isolated tissues, probably through specific blockade of phosphodiesterase type 5. T-1032 would be a useful compound to examine the physiologic functions of phosphodiesterase type 5 in mammalian tissues.

Keywords: Phosphodiesterase type 5 inhibitor; Aorta, rat; Corpus cavernosum, rabbit; Relaxation; Cyclic nucleotide

1. Introduction

Guanosine 3′5′-cyclic monophosphate (cGMP) is a second messenger in cellular signal transduction, and it plays a crucial role in the regulation of many physiologic functions including smooth muscle relaxation, neutrophil degranulation, inhibition of platelet aggregation, initiation of visual signal transduction, motility in spermatozoa, and development of testicular germ cells (Waldman and Murad, 1987; Furchgott and Vanhoutte, 1989; Ahlner et al., 1991; Corbin et al., 1993; Hobbs and Ignarro, 1996; Moro et al., 1996; Lohmann et al., 1997; Middendorff et al., 2000). Intracellular cGMP levels are regulated by a balance between the rate of synthesis by guanylyl cyclase and the rate of hydrolysis by cGMP-specific phosphodiesterases (Beavo, 1995; Soderling and Beavo, 2000). Among cGMP-specific phosphodiesterases (type 5, type 6, and type 9), phosphodiesterase type 5 is localized to some specific tissues including vascular smooth muscles, trachea, pulmonary artery, platelets, and corpus cavernosum (Beavo, 1995; Polson and Strada, 1996; Moreland et al., 1998; Wallis et al., 1999). Therefore, the inhibition of this enzyme is expected to induce an increase in cGMP levels and to affect physiologic functions in these tissues.

Recently, T-1032 (methyl-2-(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinoline carboxylate sulfate), a newly synthesized isoquinoline derivative (Fig. 1), was reported to produce a specific inhibition of phosphodiesterase type 5 in an enzyme assay (Kotera et al., 2000). The 50% inhibitory concentration (IC_{50} value) is 1.0 ± 0.12 nM for the purified enzyme obtained from canine lung. The selec-

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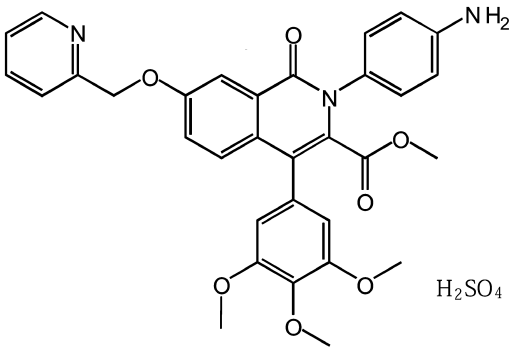

Fig. 1. Chemical structure of T-1032.

tivity for of phosphodiesterase type 5 over other phosphodiesterases (type 1, type 2, type 3, type 4) is 3000-fold or more, and the selectivity over phosphodiesterase type 6 is approximately 30-fold. The potency and selectivity of T-1032 are similar to those of sildenafil. To date, however, no evidence has been obtained whether T-1032 displays pharmacologic functions as a specific phosphodiesterase type 5 inhibitor in mammalian tissues. In this study, therefore, we examined the properties of T-1032 as a specific phosphodiesterase type 5 inhibitor in the isolated rat aorta and rabbit corpus cavernosum.

2. Materials and methods

This study was approved by the Animal Research Committee of Tanabe Seiyaku and all efforts were made to minimize animal suffering and to reduce the number of animals.

2.1. Isometric tension and cyclic nucleotide levels in rat aorta

Male Wister rats weighing 180 to 250 g (8 to 10 weeks old) were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and killed by exsanguination. The thoracic aorta was removed immediately and stored in physiological salt solution (PSS-1) at 4°C. The aorta (o.d. 2.0–2.5 mm) was cut into ring segments of 5 mm in length. The ring segment was suspended in a 10-ml organ bath containing PSS-1, which was maintained at 37 ± 0.5°C and aerated with 95% O_2 and 5% CO_2. The resting tension was adjusted to 1.5 g.

After equilibration for 60 min, 40 mM KCl was added to the preparation for characterization of the contractility. After the high KCl PSS-1 was replaced by normal PSS-1, phenylephrine (3×10^{-6} M) was added to the preparation in order to obtain a tonic contraction. T-1032 (3×10^{-11} to 3×10^{-7} M), sodium nitroprusside (3×10^{-11} to 10^{-7} M) or isoproterenol (3×10^{-9} to 3×10^{-6} M) was added cumulatively to the preparation during the phenylephrine-induced tonic contraction. When the influence of N^G-nitro-L-arginine methyl ester (L-NAME) or 1*H*-[1,2,4] oxadiazolo[4,3-α] quinoxalin-1-one (ODQ) on the T-1032-and SNP-induced relaxation was evaluated, L-NAME (10^{-3} M) or ODQ (10^{-5} M) was added to the preparation 20 min before addition of phenylephrine. When the effect of T-1032 on SNP- and isoproterenol-induced relaxation was evaluated, T-1032 (10^{-9}, 10^{-8} M) was added to the preparation 20 min before a concentration–response curve for sodium nitroprusside (3×10^{-11} to 10^{-7} M) or isoproterenol (3×10^{-9} to 3×10^{-6} M) was obtained. In some preparations, the endothelium was removed by gently rubbing the intimal surface with a cotton bar. The preservation of endothelial function or the effectiveness of endothelial removal was tested routinely by checking the responsiveness to acetylcholine (10^{-6} M), which elicited relaxation in the preparation precontracted with phenylephrine. At the end of experiment, papaverine (10^{-4} M) was added to the preparation in order to characterize the myogenic tone.

In the study of cyclic nucleotide levels (cGMP and cAMP), the arterial ring segment was suspended in the organ bath and a phenylephrine (3×10^{-6} M)-induced contraction was obtained as described above. When T-1032 (10^{-8}, 10^{-7} M) caused a maximal change in tension, the ring segment was frozen immediately in liquid N_2. The frozen tissue was homogenized with a microhomogenizer in 1 ml of 6% trichloroacetic acid containing 1 mM EDTA. After centrifugation (5000 rpm, for 15 min, 4°C), the supernatant was extracted with water-saturated diethyl ether, and aliquots of the aqueous phase were lyophilized to dryness, and then reconstituted in 1 ml of 50 mM sodium acetate buffer (pH 6.2). The cyclic nucleotide levels in the solution were measured with commercially available cGMP and cAMP immunoassay kits (Amersham, UK). The protein contents in the pellet were measured with the bicinchoninic acid (BCA) protein assay kit (Pierce, Rochford, IL, USA). The cyclic nucleotide levels are expressed in pmol per mg protein. In this experiment, papaverine was not added to the preparation because papaverine could influence cyclic nucleotide levels (Demesy-Waeldele and Stoclet, 1977). The composition of PSS-1 was as follows (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, dextrose 11.0 (pH 7.3 or 7.4).

2.2. Electrical field stimulation in rabbit corpus cavernosum

Male New Zealand white rabbits weighing 2.8 to 4.0 kg (17 to 20 weeks old) were anesthetized with intravenously injected sodium pentobarbital (50 mg/kg) and killed by exsanguination. The preparation of corpus cavernosum (about $3 \times 3 \times 5$ mm) was obtained carefully from the enveloping tunica albuginea and stored in PSS (PPS-2) at 4°C. The preparation was suspended between electrical stimulating electrodes in a 10-ml organ bath containing

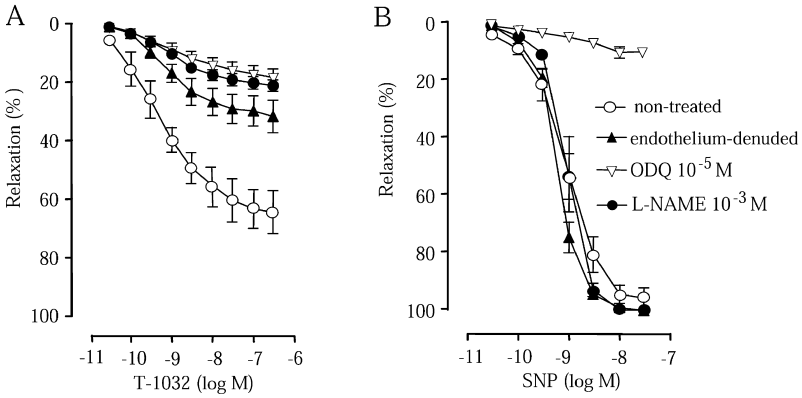

Fig. 2. Effects of L-NAME, ODQ, and endothelium-denudation on T-1032- and sodium nitroprusside-induced relaxation of the isolated rat aorta precontracted with phenylephrine. (A) Effect on T-1032-induced relaxation. (B) Effect on sodium nitroprusside-induced relaxation. T-1032 (3×10^{-11} to 3×10^{-7} M) or sodium nitroprusside (3×10^{-11} to 3×10^{-8} M) was added to endothelium-intact and endothelium-denuded preparations precontracted with phenylephrine (3×10^{-6} M). L-NAME (10^{-3} M) or ODQ (10^{-5} M) was added to the endothelium-intact preparation 20 min before the addition of phenylephrine. Maximal relaxation was taken as the papaverine (10^{-4} M)-induced relaxation. Data are shown as means ± S.E.M. for four preparations. L-NAME: N^{G}-nitro-L-arginine methyl ester, ODQ: 1*H*-[1,2,4] oxadiazolo[4,3-α] quinoxalin-1-one, SNP: sodium nitroprusside.

PSS-2 with atropine (10^{-6} M) and guanethidine (5×10^{-6} M), which was maintained at 37 ± 0.5°C and aerated with 95% O_2 and 5% CO_2. The resting tension was adjusted to 1.5 g. The gap between the preparation and the electrodes was wide enough to allow an undisturbed mechanical response and sufficiently narrow to stimulate intramural nerve terminals effectively.

After equilibration for 60 min, a train of 0.2-ms square-wave pulses (2 to 16 Hz, for 40 s) were transmurally applied with an electronic stimulator (SEN-3301, Nihon Kohden, Tokyo Japan) at an interval of 3 min to the phenylephrine (5×10^{-6} M)-precontracted preparation. Tetrodotoxin (10^{-6} M) or L-NAME (10^{-4} M) was added to the preparation under these stimulatory conditions. L-arginine (5×10^{-4} M) was added to the preparation in the presence of L-NAME (10^{-4} M) in order to examine its role as a substrate for nitric oxide (NO) synthase. When the influence of T-1032 or sildenafil (10^{-9} to 10^{-6} M) on the electrical field stimulation-induced relaxation was examined, the stimulatory condition was selected by changing the frequency (1 to 16 Hz, for 40 s, at an interval of 10 min) in order to obtain approximately 10% relaxation of the phenylephrine-precontracted preparation. The composition of PSS-2 was as follows (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, dextrose 11.0, EDTA-2 Na 0.023 (pH 7.3 or 7.4).

2.3. Statistical analysis

Statistical analysis was performed by using Dunnett's test (versus vehicle in (Figs. 3, 4 and 6)). A difference was considered significant when the *P* value was less than 0.05. EC_{50} values (Fig. 4) and EC_{30} values (Fig. 6B) were calculated from each concentration–response curve. EC_{30} values were chosen in Fig. 6B because both T-1032 and sildenafil did not reach 50% relaxation to papaverine-induced relaxation in some preparations.

2.4. Chemicals

T-1032 (methyl-2-(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinoline carboxylate sulfate) and sildenafil citrate were synthesized by Discovery Research Laboratory, Tanabe Seiyaku (Saitama, Japan). ODQ was purchased from Tocris Cookson (Ballwin, MO, USA). L-NAME, L-phenylephrine hydrochloride, papaverine hydrochloride, guanethidine sulfate, and acetylcholine were purchased from Sigma (St. Louis, MO, USA). L-arginine was purchased from Peptide

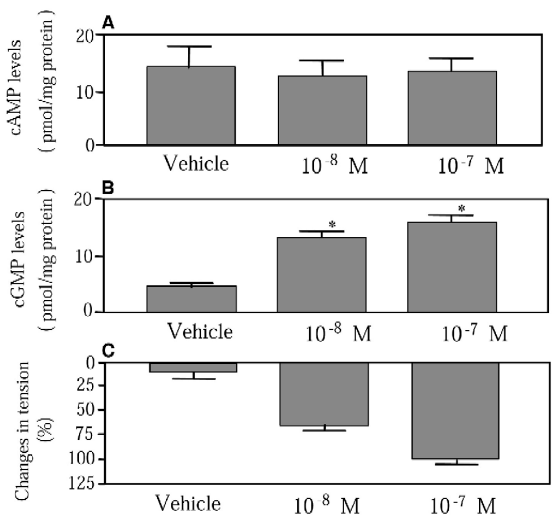

Fig. 3. Changes in cyclic nucleotide levels and isometric tension by treatment with T-1032 in isolated rat aorta. (A) cAMP levels. (B) cGMP levels. (C) Changes in tension. T-1032 (10^{-8}, 10^{-7} M) was added to preparations precontracted with phenylephrine (3×10^{-6} M). After the maximal change in tension was recorded, the preparation was frozen immediately in liquid N_2. Cyclic nucleotide (cAMP and cGMP) levels were measured with a commercially available kit (see Section 2). Data are shown as means ± S.E.M. for five preparations. The changes in tension are expressed as percentages of the maximal contraction induced by phenyrephrine.

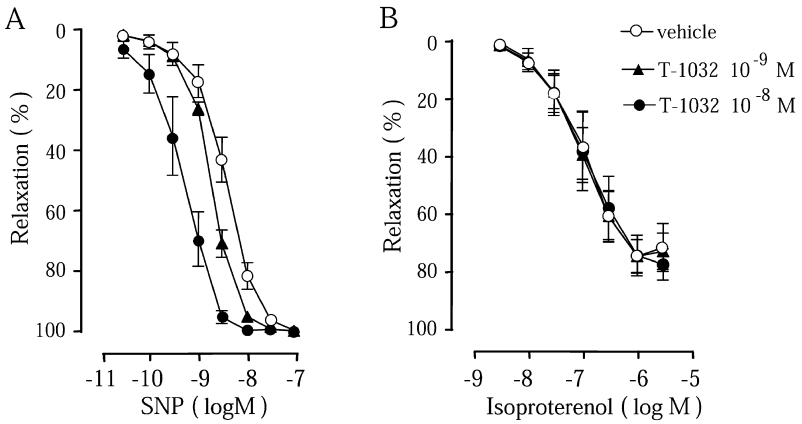

Fig. 4. Effects of T-1032 on the concentration–response curve for sodium nitroprusside and isoproterenol in the isolated rat aorta precontracted with phenylephrine. (A) Effects on concentration–response curve for sodium nitroprusside. The 50% effective concentration (EC_{50}) values of vehicle, 10^{-9} M T-1032, and 10^{-8} M T-1032 were 3.5×10^{-9}, 1.8×10^{-9}, 5.0×10^{-10} M, respectively. A significant difference was observed between vehicle- and 10^{-8} M T-1032-treated groups ($P < 0.05$). (B) Effects on concentration–response curve for isoproterenol. EC_{50} values of vehicle, 10^{-9} M T-1032, and 10^{-8} M T-1032 were 1.7×10^{-7}, 1.7×10^{-7}, 1.8×10^{-7} M, respectively. T-1032 (10^{-9} M, 10^{-8} M) was added 20 min before the concentration–response curve for sodium nitroprusside (3×10^{-11} to 10^{-7} M) or isoproterenol (3×10^{-9} to 3×10^{-6} M) was recorded. Maximal relaxation was taken as the papaverine (10^{-4} M)-induced relaxation. Data are shown as means ± S.E.M. for four preparations. SNP: sodium nitroprusside.

Institute (Osaka, Japan). Sodium nitroprusside and DL-isoproterenol hydrochloride were purchased from Nacalai Tesque (Kyoto, Japan). Tetrodotoxin and atropine sulphate monohydrate were purchased from Wako (Osaka, Japan). All other chemicals were of analytical grade.

3. Results

Fig. 2 shows the vasorelaxing properties of T-1032 and sodium nitroprusside in the isolated rat aorta. T-1032 (3×10^{-11} to 3×10^{-7} M) caused a concentration-dependent relaxation in the isolated rat aorta precontracted with phenylephrine. The relaxation reached a plateau at a concentration of 10^{-7} M (Fig. 2A). The relaxation at 10^{-7} M was 63.4 ± 6.6% of the papaverine-induced relaxation. The T-1032-induced vasorelaxation was attenuated not only by a removal of the endothelium but also by pretreatment with L-NAME (10^{-3} M) or ODQ (10^{-5} M). The attenuation by L-NAME and ODQ was almost the same as that in the endothelium-denuded preparation (L-NAME-treated: 20.3 ± 2.1%, ODQ-treated: 17.2 ± 2.8%, endothe-

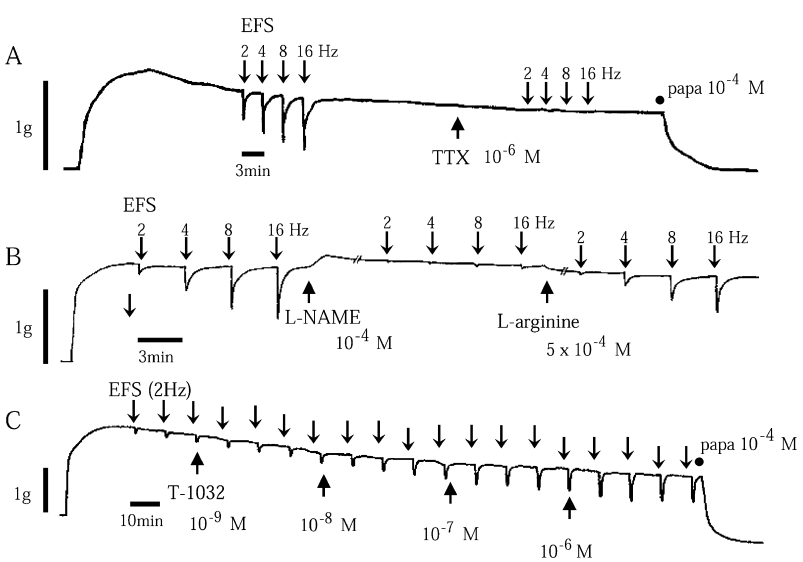

Fig. 5. Typical tracings showing the characteristics of EFS-induced relaxation in the isolated rabbit corpus cavernosum precontracted with phenylephrine. (A) Effect of tetrodotoxin on electrical field stimulation-induced relaxation. (B) Effect of L-NAME on electrical field stimulation-induced relaxation and influence of L-arginine on the L-NAME-inhibited relaxation. (C) Effect of T-1032 on electrical field stimulation-induced relaxation. EFS: electrical field stimulation, TTX: tetrodotoxin, L-NAME: N^G-nitro-L-arginine methyl ester, papa: papaverine.

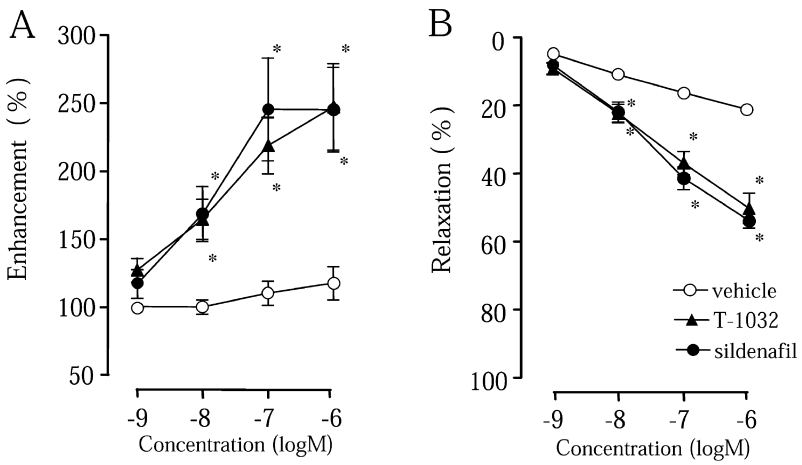

Fig. 6. Effect of T-1032 on electrical field stimulation-induced relaxation and basal tension in the isolated rabbit corpus cavernosum precontracted with phenylephrine. (A) Effect on electrical field stimulation-induced relaxation. The electrical field stimulation-induced relaxation in the absence of T-1032 was expressed as 100%. (B) Effect on basal tension. Maximal relaxation was taken as the papaverine (10^{-4} M)-induced relaxation. Data are shown as means ± S.E.M. for eight preparations. * Significant difference between vehicle-treated and drug-treated groups at $P < 0.05$.

lium-denuded: 29.9 ± 5.2%, at a concentration of 10^{-7} M T-1032) (Fig. 2A). In contrast, the sodium nitroprusside-induced relaxation was attenuated by pretreatment with ODQ, but not by L-NAME or removal of the endothelium (Fig. 2B). The SNP-induced maximal relaxation was 96.4 ± 3.6% of the papaverine-induced relaxation.

Fig. 3 shows the influence of T-1032 on cyclic nucleotide levels and basal tension in the phenylephrine-precontracted rat aorta. T-1032 (10^{-8}, 10^{-7} M) caused an increase in cGMP levels but not in cAMP levels with a reduction in basal tension. The increase in cGMP levels by T-1032 was approximately threefold at a concentration of 10^{-7} M (vehicle-treated: 4.4 ± 0.4 pmol/mg protein, T-1032-treated: 14.6 ± 2.0 pmol/mg protein).

Fig. 4 shows the effect of T-1032 on the vasorelaxation induced by sodium nitroprusside and isoproterenol in the isolated rat aorta precontracted with phenylephrine. T-1032 (10^{-9}, 10^{-8} M) caused a leftward shift of the concentration–response curve for sodium nitroprusside (3×10^{-11} to 10^{-7} M) in a concentration-dependent manner (Fig. 4A). The EC_{50} values of vehicle, 10^{-9} M T-1032, and 10^{-8} M T-1032 were 3.5×10^{-9} M, 1.8×10^{-9} M, 5.0×10^{-10} M, respectively. In contrast, T-1032 did not influence the concentration–response curve for isoproterenol (3×10^{-9} to 3×10^{-6} M) at the same concentrations (Fig. 4B).

Fig. 5 shows typical tracings of the influence of T-1032 on electrical field stimulation-induced relaxation in the isolated rabbit corpus cavernosum. Electrical field stimulation (2 to 16 Hz) caused a frequency-dependent relaxation in the isolated rabbit corpus cavernosum precontracted with phenylephrine. The relaxation was attenuated by pretreatment with tetrodotoxin (10^{-6} M) or L-NAME (10^{-4} M). The L-NAME-inhibited relaxation was restored by excess amounts of L-arginine (5×10^{-4} M) (Fig. 5B). T-1032 (10^{-9} to 10^{-6} M) caused a potentiation of the electrical field stimulation-induced relaxation, and this potentiation was accompanied by a decrease in basal tension (Fig. 5C).

Fig. 6 shows the concentration–response curve for T-1032 and sildenafil on the potentiation of electrical field stimulation-induced relaxation and on basal tension in the phenylephrine-precontracted rabbit corpus cavernosum. T-1032 (10^{-9} to 10^{-6} M) produced a concentration-dependent potentiation of electrical field stimulation-induced relaxation (Fig. 6A). The activity of T-1032 at 10^{-7} M was almost the same as that of sildenafil (vehicle-treated: 109.4 ± 8.9%, T-1032-treated: 217.9 ± 20.8%, sildenafil-treated: 244.5 ± 37.7%). Both T-1032 and sildenafil caused a reduction of basal tension to the same extent in a concentration-dependent manner (Fig. 6B). The EC_{30} values of T-1032 and sildenafil for the relaxation were 4.0×10^{-8} M and 2.5×10^{-8} M, respectively. The relaxation produced by T-1032 and sildenafil at a concentration of 10^{-7} M was 37.4 ± 3.4% and 41.8 ± 3.4%, respectively.

4. Discussion

T-1032 has been shown to inhibit phosphodiesterase type 5 specifically in an enzyme assay (Kotera et al., 2000). However, to date, no evidence has been obtained whether T-1032 exerts pharmacological functions as a specific phosphodiesterase type 5 inhibitor in mammalian tissues. In this study, therefore, we examined the properties of T-1032 in the isolated rat aorta and rabbit corpus cavernosum. The present study clearly indicated that T-1032 had a specific phosphodiesterase type 5 inhibitory action in mammalian tissues.

In the isolated rat aorta, the sodium nitroprusside-induced vasorelaxation was attenuated by ODQ, a guanylyl

cyclase inhibitor (Garthwaite et al., 1995). The relaxation was attenuated neither by L-NAME, a NO synthase inhibitor (Rees et al., 1990), nor by removal of the endothelium. These results, which were consistent with previous reports (Lincoln and Fisher-Simpson, 1984; Sobey and Faraci, 1997; Van der Zypp and Majewski, 1998), indicated that sodium nitroprusside directly activated guanylyl cyclase in vascular smooth muscle and caused the resulting vasorelaxation. The T-1032-induced vasorelaxation was endothelium-dependent, and the vasorelaxation was attenuated by both ODQ and L-NAME. These results suggested that the T-1032-induced vasorelaxation was associated with NO or NO-related substance(s) released from the endothelium. In order to examine whether the T-1032-induced vasorelaxation is specifically related to the NO/cGMP signaling pathway, we performed two additional experiments with isolated rat aorta: (1) measurement of cGMP and cAMP levels, and (2) influence of T-1032 on cGMP- and cAMP-associated vasorelaxation. In the former experiment, cGMP levels, but not cAMP levels, were elevated in T-1032-treated vessels. In the latter experiment, T-1032 caused a potentiation of the vasorelaxation induced by sodium nitroprusside, a NO donor (Ignarro et al., 1991), but not of the vasorelaxation induced by isoproterenol, which mainly activates the production of cAMP (Kukovetz et al., 1981). These results suggested that T-1032 amplified the NO/cGMP signaling pathway and resulting vasorelaxation. As phosphodiesterase type 5 is a hydrolytic enzyme for cGMP and is abundantly present in vascular smooth muscle (Beavo, 1995; Polson and Strada, 1996), the inhibition of phosphodiesterase type 5 theoretically leads to the accumulation of cGMP in the vascular smooth muscle. The results for the isolated rat aorta satisfied the circumstantial evidence that T-1032 has a specific phosphodiesterase type 5 inhibitory action in mammalian tissues.

Phosphodiesterase type 5 is also abundantly present in the penile corpus cavernosum (Boolell et al., 1996; Taher et al., 1997; Ballard et al., 1998; Moreland et al., 1998). In this tissue, NO released from nitrergic nerves in the walls of arteries and sinusoids appears to play a major role in the relaxation of the corpus cavernosal smooth muscle during sexual stimulation (Andersson and Wagner, 1995; Bush et al., 1992; Holmquist et al., 1992; Pickard et al., 1995). This physiological process is a reason why sildenafil, a specific phosphodiesterase type 5 inhibitor, causes a potentiation of penile erection during sexual stimulation (Corbin and Francis, 1999; Langtry and Markham, 1999). Therefore, we used penile tissue to evaluate the potency of PDE5 inhibitors. In the isolated rabbit corpus cavernosum, we first confirmed whether the electrical field stimulation-induced relaxation could be attributed to NO released from nitrergic nerves. Treatment with tetrodotoxin inhibited the electrical field stimulation-induced relaxation, suggesting that the electrical field stimulation-induced relaxation was a neurogenic response. Furthermore, the treatment with L-NAME inhibited the electrical field stimulation-induced relaxation and the L-NAME-inhibited relaxation was restored by treatment with excess L-arginine, suggesting that the electrical field stimulation-induced relaxation was due to NO or NO-related substance(s). These results suggested that the electrical field stimulation-induced relaxation was associated with NO released from nitrergic nerves, which is consistent with previous reports (Hayashida et al., 1996; Ballard et al., 1998; Ayajiki et al., 1998). Under these experimental conditions, T-1032 as well as sildenafil produced a potentiation of electrical field stimulation-induced relaxation. This result suggested that T-1032, like sildenafil, would amplify the neuronal NO/cGMP signaling pathway by phosphodiesterase type 5 inhibition, and resulting in potentiation of relaxation in the corpus cavernosum. Taken together with the results for the isolated rat aorta, T-1032 thus would have potentiating effects on both the vascular and the neuronal NO/cGMP signaling pathway in the isolated mammalian tissues. The present study in the isolated rat aorta and rabbit corpus cavernosum clearly indicated that T-1032 had characteristics as a specific phosphodiesterase type 5 inhibitor in mammalian tissues.

In the isolated rat aorta, the T-1032-induced maximal vasorelaxation was modest compared with the SNP-induced one (Fig. 2A vs. B). When the maximal vasorelaxation was reached with 10^{-7} M T-1032, cGMP levels were approximately threefold higher than basal levels (Fig. 3). Although we did not measure cGMP levels in sodium nitroprusside-treated preparations, the cGMP levels are reported to be elevated 10-fold or more in sodium nitroprusside (3×10^{-6} M or more)-treated rat aorta (Rapoport et al., 1985; Tsai et al., 1989). Therefore, the difference in maximal relaxation between T-1032 and sodium nitroprusside appeared to be attributed to the cGMP levels in the vessels. As other phosphodiesterases (type1, type 3, and type 10) are localized in these vessels (Vemulapalli et al., 1996; Maurice, 1998; Nagaoka et al., 1998; Kotera et al., 1999; Wallis et al., 1999), these phosphodiesterases may hydrolyze cGMP and keep cGMP levels relatively low in the vessel. Further studies will be required in order to clarify the difference in the maximal relaxation.

It has been reported that sildenafil causes a potentiation of electrical field stimulation-induced relaxation without causing changes in basal tension in the isolated human corpus cavernosum (Ballard et al., 1998). However, sildenafil as well as T-1032 produced a reduction in basal tension in the phenylephrine-precontracted corpus cavernosum (Fig. 6A). In our preliminary study, the T-1032-induced relaxation was attenuated by pretreatment with L-NAME or ODQ (unpublished observations). Therefore, the relaxation could be associated with potentiation of the NO/cGMP signaling pathway. As some amounts of NO would be released spontaneously by the endothelial cells lining the vessels and sinusoids of the corpus cavernosum, both T-1032 and sildenafil could cause relaxation through an elevation of basal cGMP levels by blockade of phosphodiesterase type 5. The discrepancy between our results

and those of other researchers may be due to experimental conditions.

It is also of interest that the T-1032-induced relaxation was more marked in the rat aorta than in the rabbit corpus cavenosum (Fig. 2A vs. Fig. 6A). Although we did not explore the reason for this difference, the result suggested that T-1032 had a more profound influence on cardiovascular tissue than on penile tissue under basal conditions. Recently, we confirmed that T-1032 had a potent venodilator property, causing modest hypotension, in anesthetized rats (Inoue et al., 2000) and dogs (Yano et al., 2000). In addition, Fujii et al. (1999) have reported that acute intravenous infusion of T-1032 improves heart failure condition in cardiac pacing dogs, probably through an accumulation of cGMP levels, which are retained by endogenous natriuretic peptide. Although phosphodiesterase type 5 inhibitors have been developed as a drug for male erectile dysfunction, phosphodiesterase type 5 inhibitors, including T-1032, may influence hemodynamics and have potential for the treatment of cardiovascular diseases. Detailed studies will be essential to find a new clinical indication for specific phosphodiesterase type 5 inhibitors.

In conclusion, we confirmed that T-1032 had properties as a specific PDE5 inhibitor in mammalian tissues. T-1032 would be a useful compound to examine the physiologic functions of PDE5 and its relevance to human diseases.

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