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A Comparison of the Relaxation Responses of Isolated Cavernosal Smooth Muscles by Endothelium-independent and Endothelium-dependent Vasodilators in Diabetic Men with Impotence

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This study was intended to explore the effects of endothelium-independent, direct smooth muscle relaxants(papaverine, verapamil) and endothelium-dependent vasodilators(acetylcholine, bradykinin, adenosine) on the isolated cavernosal smooth muscle strips taken from diabetic men with impotence. When smooth muscle contraction was evoked with norepinephrine for the study of relaxation to these vasodilators, the tension induced was similar in diabetic and non-diabetic men with impotence. Papaverine showed the strongest relaxation response followed by verapamil, acetylcholine, bradykinin and adenosine both in non-diabetic and in diabetic men. Relaxation of the cavernosal tissues to endothelium-independent vasodilators was similar in non-diabetic and diabetic men. However, the relaxation response of the tissues to endothelium-dependent vasodilators was significantly reduced in the diabetic group compared with that in the non-diabetic group ($p<0.05$). In conclusion, the impairment of endothelium-mediated relaxation of cavernosal smooth muscle seems to play a more important role in the pathogenesis of diabetogenic impotence rather than the problem of smooth muscle itself. This finding forms a rational basis for the use of intracavernosal injections of vasodilators to induce endothelium-independent relaxation of the cavernosal smooth muscle in the patients with diabetogenic impotence.

Key Words : *Diabetes mellitus, Impotence, Cavernosal smooth muscle, Endothelium-dependent vasodilator.*

INTRODUCTION

Diabetes mellitus is one of the most common cause of organic impotence. Both vasculogenic (Herman et al., 1978 ; Jevtich et al., 1985) and neurogenic (Kolodny et al., 1974 ; Saenz de Tejada and Goldstein, 1988) causes have been considered among the major etiological factors in diabetogenic impotence. However, cavernosal smooth muscle atrophy and replacement of the smooth muscle by fibrotic tissue have been found in diabetic men with impotence (Fani et al., 1983 ; Lee et al.,

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1991), and diabetes can fundamentally alter the properties of the trabeculae, thereby impairing their relaxation and thus the erectile process.

Many diabetic patients have a good response to intracavernosal pharmacotherapy with direct smooth muscle relaxants compared with severity of the diabetes mellitus and the erectile dysfunction, which suggests that other factors rather than myogenic ones may play an important role in the pathogenesis of diabetogenic impotence. In this respect, we intended this study to compare the effects of endothelium-independent, direct smooth muscle relaxants and endothelium-dependent vasodilators on the isolated cavernosal smooth muscle strips of diabetic men with impotence.

MATERIALS AND METHODS

The study subjects were 19 men who underwent the implantation of penile prosthesis for the treatment of erectile dysfunction. Five of the patients, whose ages ranged from 35 to 64 years (mean 52), had a history of diabetes mellitus. The control population, whose age ranged from 19 to 71 years (mean 49.7), consisted of 14 patients with erectile dysfunction who had no history of diabetes mellitus and who had normal blood glucose levels at the time of admission for surgery. One of the control patients had hypertension but none among the diabetic patients had hypertension.

Specimens of the cavernosal tissue were obtained from the both sides of corpora cavernosa at the time of the implantation of penile prosthesis in the non-diabetic and diabetic patients. The removed cavernosal tissue was immediately placed in oxygen-saturated HEPES-buffered physiological salt solution (NaCl, 140mM ; KCl, 5mM ; $CaCl_2$, 2mM ; $MgCl_2$, 1mM ; HEPES, 5mM ; glucose, 11mM, pH titrated with 1N NaOH to 7.4) and studied within one hour. Among the 38 specimens obtained, 10 from the diabetic patients and 19 from the non-diabetic patients were used for this study. The cavernosal strips were trimmed to a size of 0.2 by 0.2 by 1.0 cm and mounted. To record isometric tension, the cavernosal strips were fixed with silk tie to an electrode support on one end and to a wire connected force transducer (52-9545, Harvard, U.S.A.) and polygraph (50-8630, Harvard, U.S.A.) on the other end. The tissue was placed in 30 ml organ chamber and bathed in physiological salt solution of the following composition in mM : NaCl, 116 ; KCl, 5 ; $CaCl_2$, 2 ; $MgCl_2$, 1 ; $NaHCO_3$ 24, glucose, 11. This solution was bubbled with a gas of 95% O_2, 5% CO_2 and maintained at 37℃, pH 7.4. The resting tension was adjusted to the optimal isometric tension for the contraction of each strip by determining the tension at which contraction by norepinephrine was maximal.

Relaxation responses induced by endothelium-independent vasodilators, papaverine hydrochloride (Aldrich, U.S.A.) and verapamil (Sigma, U.S.A.) and those induced by endothelium-dependent vasodilators, acetylcholine (Sigma, U.S.A.), bradykinin (Sigma, U.S.A.) and adenosine (Sigma, U.S.A.) were then studied in cavernosal strips in which tone was elicited by norepinephrine (10^{-6} M). Concentration-response curves were determined by adding cumulative increments of the dilator agents from 10^{-9} to 10^{-4} M (except bradykinin of 10^{-10} to 10^{-5} M) to the chamber.

Contractile tension was expressed in grams. Relaxation was expressed as a percentage of the maximal relaxation (tension at maximal relaxation by the dilator/tension at maximal contraction by norepinephrine of 10^{-6} M). The concentration response curves in the diabetic and non-diabetic patients were compared by Student's unpaired t-test.

RESULTS

When the contraction of the cavernosal strips was induced by norepinephrine in cumulative manner from 10^{-9} to 10^{-4} M, half concentration of the maximum effect was about 10^{-6} M. Thus, we used this concentration as a standard one to induce constant tone before exposure to the vasodilator agents. The tension induced by norepinephrine was similar in the diabetic and the non-diabetic men. The most potent agent to relax the cavernosal strip in the same condition was papaverine, followed by verapamil, acetylcholine, bradykinin and adenosine in order in the tisssues from both non-diabetic and diabetic men compared with the relaxing magnitude at same concentration of each drug (data not shown).

The acetylcholine (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4})-induced relaxation of the cavernosal strip was significantly less pronounced in the tissue from diabetic patients than in the tissue from non-diabetic patients ($p<0.05$). The adenosine (10^{-8} to 10^{-6} M) and bradykinin (10^{-10} to 10^{-5} M)-induced relaxations of the cavernosal smooth muscles were also signifi-

cantly less pronounced in the tissue from diabetic patients than in the tissue from non-diabetic patients ($p<0.05$) (Table 1, Fig. 1, 3). Among the three endothelium-dependent vasodilators, bradykinin showed statistically least conspicious relaxation in the tissues from diabetic patients than in the tissues from non-diabetic patients. However, there was no significant difference in the relaxation of the cavernosal strips induced by papaverine (10^{-9} to 10^{-4} M) or verapamil (10^{-9} to 10^{-4} M) between the tissue from diabetic patients and the tissue from non-diabetic patients (Table 2, Fig. 2, 4).

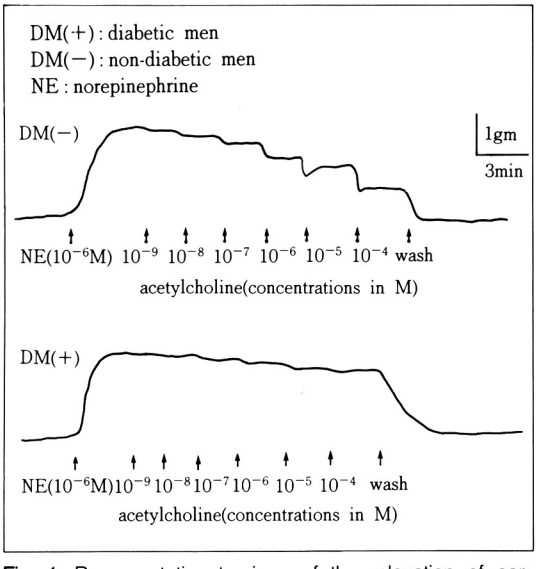

Fig. 1. Representative tracings of the relaxation of corporeal smooth muscle from a non-diabetic patient and a diabetic patient, induced by acetylcholine.

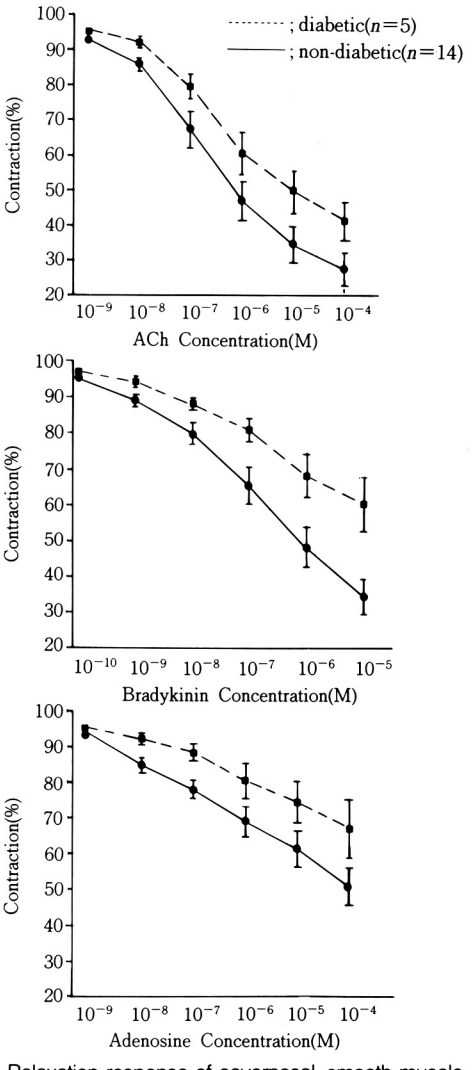

Fig. 3. Relaxation response of cavernosal smooth muscle strip of diabetic and non-diabetic patients, induced by endothelium-dependent vasodilators.

Table 1. Relaxation response of isolated cavernosal strips of endothelium-dependent vasodilators between diabetic and non-diabetic men with impotence.

Vasodilator		Concentrations(M)					
		$10^{-9}(10^{-10})$	$10^{-8}(10^{-9})$	$10^{-7}(10^{-8})$	$10^{-6}(10^{-7})$	$10^{-5}(10^{-6})$	$10^{-4}(10^{-5})$
Acetylcholine	DM(−)	92.86±1.32	85.77±1.92	67.43±5.20	47.32±5.62	35.08±5.11	28.00±4.63
	DM(+)	95.57±0.85*	92.11±1.84*	79.67±3.61*	60.69±6.16	50.25±6.19*	41.85±5.44*
Adenosine	DM(−)	94.12±0.60	85.39±1.93	78.48±2.45	69.71±4.27	62.22±5.06	51.78±5.45
	DM(+)	95.60±0.78	92.61±1.49**	88.88±2.52**	81.12±5.04*	75.22±5.89*	67.83±8.29
Bradykinin	DM(−)	95.09±0.71	89.08±1.59	80.06±3.01	65.85±5.06	48.41±5.85	34.79±5.02
	DM(+)	97.07±0.43	94.23±0.59**	88.06±1.68*	80.88±3.19*	68.62±5.89*	60.64±7.47**

Concentrations in the parentheses indicate the concentration of bradykinin. Values are mean±SEM(%).
Number of DM(+) ; 5, Number of DM(−) : 14, *$p<0.05$, **$p<0.01$

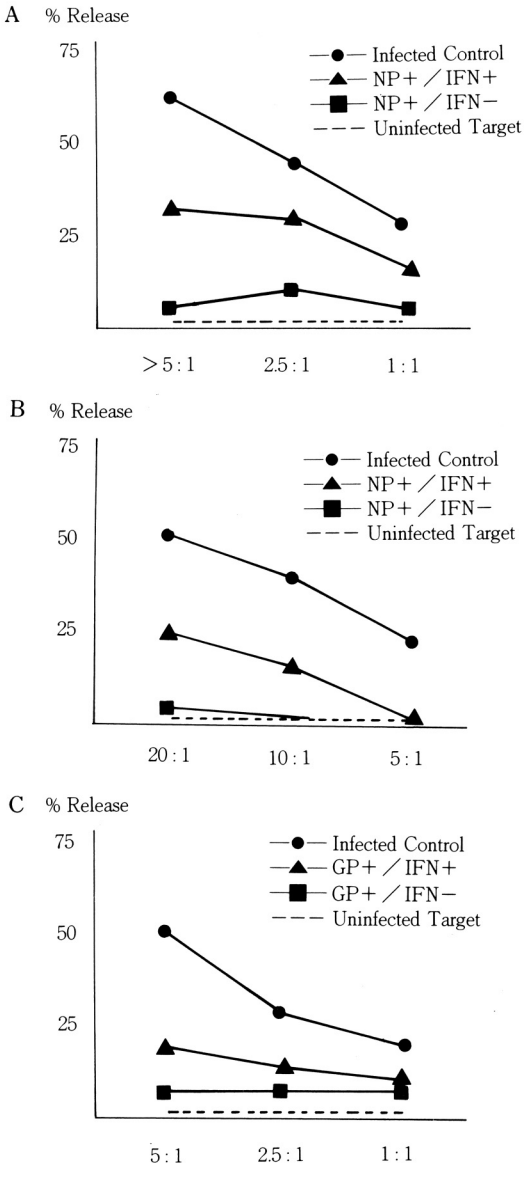

Fig. 1. Secondary CTL response to LCMV antigens in double transgenic mice and single transgenic littermates used as controls. Lymphocytes from NP^{+}/IFN-γ^{+} mice killed both LCMV-infected(A) and vaccinia-NP-infected targets(B) to a moderate degree, whereas lymphocytes from NP^{+}/IFN-γ^{+} mice exerted only negligible killing of these targets(A and B). These results suggest that lymphocytes from the double transgenic mice are spontaneously and specifically sensitized to LCMV-NP antigen. Lymphocytes from GP^{+}/IFN-γ^{+} mice killed a small portion of LCMV-infected targets(C). Lymphocytes from LCMV-infected mice used as positive controls showed strong CTL response to LCMV antigens(A-C).(From Lee et al., 1995 with permission)

HA mice). However, neither insulitis nor diabetes in the RIP-HA mice was observed even after infection with vaccinia-HA recombinant virus(Lo et al., 1992). This seems to be in apparent contradiction with the results obtained using LCMV transgenic mice(Ohashi et al., 1991 ; Oldstone et al., 1991), and it was speculated that the inability of vaccinia, not a mouse pathogen, to infect host cells and subsequent limitation of T cell stimulatory activity might be responsible for the deficient T cell response after virus challenge.

TCR TRANSGENIC MICE

TCR transgenic mice were initially produced to enlarge the lymphocyte population with a specific TCR by allelic exclusion and to allow the pursuit of lymphocytes specific for non-MHC molecules, which has been impossible because of the small number of those lymphocytes. TCR transgenic mice were also bred with other transgenic mice expressing the authentic antigen in the peripheral tissue.

In the case of LCMV transgenic mice which showed the evidence of peripheral ignorance, the addition of TCR transgene encoding TCR specific for LCMV antigen on over 70% of peripheral lymphocytes further demonstrated that there was no clonal deletion, abortion or a decrease in the TCR density of the lymphocytes in the double transgenic offsprings (Ohashi et al., 1991). They proposed that unprimed lymphocytes harboring TCR were not 'anergized' on the basis that cytotoxic T lymphocyte(CTL) response were similar between LCMV-transgenic and -non-transgenic mice, and only could not recognize the antigen due to the inability of pancreatic β-cells to stimulate T lymphocytes. These results further support their concept of immunological ignorance.

On the other hand, in SV40 T transgenic mice, additional expression of the transgenic SV40 T-specific TCR led to (auto)immune response to transgenic SV40 T protein and pancreatic destruction in double transgenic mice expressing the antigen on pancreatic acinar cells and TCR on the majority of lymphocytes(Geiger et al., 1992). Consistent with this result, expression of HA-specific TCR transgene from Th2 clone on RIP-HA transgenic mice showed that autoimmune response to HA antigen and autoimmune diabetes occurred, according to the strain of breeding mice(Scott et al., 1994). Additionally, TCR transgene from HA-specific Th1 cells also induced insulitis and

Adrenergic nerves cause the contraction of cavernosal smooth muscle and mediate the detumescence of the erect penis (Hedlund et al., 1984 ; Saenz de Tejada et al., 1989). Acetylcholine (Furchgott and Zawadski, 1980), adenosine (de May and Vanhoutte, 1981) and bradykinin (Kimoto et al., 1990 ; Kim and Kim, 1994) have been found to induce endothelium-dependent relaxation of cavernosal smooth muscle. Papaverine (Poech and Kukovetz, 1971 ; Kirkeby et al., 1990) and verapamil (Cho et al., 1993) are known as endothelium-independent, direct smooth muscle relaxants. Norepinephrine induces concentration-dependent contractions of isolated cavernosal tissue. Azadzoi and Saenz de Tejada (1992) reported that the tension of cavernosal strip induced by norepinephrine was similar in alloxan-induced diabetic rabbits and control rabbits. Saenz de Tejada et al (1989) reported that autonomic nerve relaxation of cavernosal smooth muscle from diabetic men with impotence was impaired, although autonomic mediated contractions were maintained. We also found there was no significant difference in the amplitude of contraction produced with norepinephrine for the study of relaxation between non-diabetic and diabetic men. This means that the adrenergic component of the cavernosal tissue is rarely impaired in diabetes. However, Melman et al (1980) reported that the noradrenaline content of the corpora cavernosa was decreased in diabetic impotent males as compared with males whose impotence had a non-neurological cause. We found endothelium-dependent relaxation was impaired, as evidenced by a lower degree of muscle relaxation after administration of acetylcholine, bradykinin and adenosine in the tissue from diabetic men than in that from non-diabetic men. Endothelium-dependent vasodilators such as acetylcholine, bradykinin and adenosine act by releasing nitric oxide from endothelial cells, and this causes smooth muscle relaxation via an increase in intracellular cyclic GMP content. However, endothelium-independent relaxation after the administration of papaverine and verapamil was similar in tissues from the diabetic and non-diabetic men. These findings suggest that the impairment of endothelium-mediated relaxation of cavernosal smooth muscle seems to play a more important role in the pathogenesis of diabetogenic impotence rather than problems of the smooth muscle itself. Thus, this may provide a rationale for the treatment of diabetogenic impotence by intracavernosal injection of vasodilators to induce endothelium-independent relaxation of the smooth muscle.

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