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ESTIMATION OF NOR-ADRENALINE CONTENT OF HUMAN PENILE TISSUE IN DIABETIC MEN WITH/WITHOUT NEUROPATHY

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ABSTRACT

Penile tissue consisting of corps cavernosum (cc) and tunica albuginea (TA) was obtained from 35 impotent patients undergoing surgery for implantation of penile prostheses and was examined for nor adrenaline content. 10 patients were classified as a non diabetic non neuropathic group, on the basis of their clinical history and differential diagnostic symptoms which included Peyronie's disease, vascular disease, hypertension and psychogenic impotence. The nor adrenaline content was found to be significantly lower in tunica albuginea than the corpus cavernosum ($P<0.02$) in this group. The nor adrenaline content of corpus cavernosum from insulin dependent (IDDM) and non insulin dependent (NIDDM) diabetic neuropathic patients was also found to be significantly lower ($P<0.02$) than that of non diabetic non neuropathic patients. The nor adrenaline content of tunica albuginea however, was similar in both groups. A non significant association in the content of nor adrenaline in corpus cavernosum and tunica albuginea among IDDM and NIDDM diabetic neuropathics was also observed. These results provide evidence that an underlying ncuropathic factor itself causes vascular as well as metabolic changes in the adrenergic nerves of the penis in diabetics due to neuropathy in addition to the effect of the disease and thus may contribute to the development of impotence in these patients irrespective of their type of diabetes.

Introduction

Erectile impotence has been reported to be associated with diabetes mellitus (Ali *et al.* 1993, 1993a, 1993b; Ellenberg, 1980). The etiology has been reported to be a neuropathic abnormality in the male genital organs and/or vascular changes in the cor-

pora cavernosa (Weiss, H.D, 1972). Recently it has been recognized that it is likely that both sympathetic and parasympathetic nervous system function together to produce highly specific vasodilation and vasoconstriction responses in the appropriate vasculature (Jevtich et al, 1982). In addition, the contractile state of the smooth muscle of the trabeculae of the corpus cavernosum has been implicated to have a role in both erection and detumescence and this is also under autonomic nervous control (Willis et al., 1981).

The innervation of penis is autonomic and somatic. The sympathetic and para sympathetic fibers arise from hypogastric and pelvic splanchnic nerves, respectively and are distributed in the corpora cavernosa. Normal penile erection in man depends upon the integration of the thoracolumbar and sacral afferent signals, intact autonomic nerves to penis, normal erectile tissue and adequate blood supply (Weiss H.D., 1972). Abnormalities in any one of these areas can lead to erectile dysfunction.

The increase frequency of impotence in diabetic men has long been recognized (Karacan, 1980). Several clinical studies have suggested that atherosclerosis is the major etiological factor involved in the development of impotence in diabetes (Her-men et al., 1978). However it has also been suggested that impotence in diabetic men is a manifestation of autonomic neuropathy (Faerman et a1., 1974). Both the parasympathetic (Crowe et al., 1983) and sympathetic (Melman et al., 1979) innervation have been implicated. However to date there is no clinical electro-physiological technique for the evaluation of the integrity of these nerve fibers and the assessment of a neuropathic factor has been impended by the lack of an objective laboratory test. In the recent years many histochemical fluorescence techniques have demonstrated that partly active intestinal polypeptide like VIP (Vaso active intestinal polypeptide) acts as transmitter in the nerves supplying the penile arteries (Anderson et al., 1984) and cavernous smooth muscles (GU, J., Polak et al., 1983). Electron microscopic studies have shown abnormalities in myelinated axons within the fibrous plaque while the unmyelinated axons surrounding the erectile tissue appeared unchanged, thus suggesting the involvement of autonomic innervation during the erectile impotence (Vande et al., 1981). A significant reduction in nor adrenaline levels in erectile tissue in diabetes associated with impotence has been reported previously (Melman et al., 1980) however the reduction was not found in non-insulin dependent diabetics. In another study a marked reduction was found in VIP immunoreacivity in the nerves of penis from streptozotocin diabetic rat, and an similar reduction was found in a preliminary study of one human diabetic patient with impotence (Crowe et al., 1983). More recently nor adrenaline involvement in penile detumescence has been reported in literature (Diederichs et al., 1990). Involvement of acetylcholine as a successful treatment for impaired neurogenic and endothelium mediated relaxation of penile smooth muscle from diabetic men with impotence has been reported by Saenz et al. (1989). Effect of intracavernous simultaneous injection of acetylcholine and

vasoactive intestinal polypeptide on canine penile erection has also been reported (Yoshiastu et al., 1992). These workers found an increased affinity of muscarinic receptors for acetylcholine in canine corpus cavernosum caused by VIP. The levels of nor adrenaline was also found to be higher in patients with psychogenic impotence than in normal controls and patients with vasculogenic impotence (Sae & Moon, 1992).

In the present study we have used biochemical techniques to investigate adrenergic innervation of erectile tissue in IDDM and NIDDM diabetic impotent males having an objective evidence of neuropathy.

Materials and Methods

Specimens of human penile tissue (containing both corpus cavernosum and tunica albuginea) were obtained from a series of 35 patients under going surgery for the implantation of penile prostheses. The series consisted of patients suffering from impotence due to a variety of causes including diabetes, Peyronie's disease and neuropathy. The cause of erectile dysfunction was categorized on the basis of clinical history, physical examination, nocturnal penile tumescence test, psychiatirc evaluation and additional evidence of neuropathy. Each tissue specimen was divided into several portions for analysis, which was carried out before clinical details of patients were provided. For the experimental purposes, all the patients were divided into 4 groups according to their clinical history and evidence of vascular or neurological disorder; Group I consist of 10 non diabetic patients in whom damage to autonomic nerve supply of penis was not indicated clinically as a factor in the development of impotence. It included patients where a non neuropathic case of impotence was diagnosed (Peyronie's disease). Group II consists of 5 non diabetic neuropathics with an objective evidence of abnormal bladder function (decreased urinary sensation). Group III & IV consist of 10 patients each suffering from insulin dependent diabetes mellitus (IDDM) and non insulin dependent diabetes mellitus (NIDDM) respectively, with an objective clinical evidence of peripheral and/or autonomic neuropathy. The mean ages of the four groups were similar; Group I: 53 ± 3.4 years, Group II: 50 ± 3.2 years, Group III 52 ± 5.4 years, Group IV 52 3 4.4 years.

The adrenergic innervation of all the four groups were investigated by biochemical measurement of nor adrenaline levels in the corpus cavernosum and tunica albuginea by dissecting tunica albuginea from corpus cavernosum. Both regions were frozen and stored in liquid nitrogen for biochemical assay of nor adrenaline. Levels of nor adrenaline in the tissues were measured using high performance liquid chromatography with electrochemical detection (Lincoln et al., 1987). Samples were homogenized in 500 ul of 0.1 M perchloric acid containing 0.4 mM sodium bisulphate and 12.5 ng of an internal

standard dihydroxybenzylamine (DHBA) by adding 0.1 mM ethylenediamine tetra-acetate (EDTA) in the solution used for washing the alumina. chromatography was carried out at a flow rate of 1.5 ml/min using a mobile phase consisting of 0.1 M sodium dihydrogen phosphate, 0.1 mM EDTA, 5mM heptane sulphonate (pH 5.0) containing 10% (VA') methanol on a u-Bondapak C-18 reverse phase column. Detection was accomplished with a waxy carbon paste electrode set at a potential of + 0.72 v. Quantitation was carried out measuring the peak heights of nor adrenaline and DHBA in samples and external standards. Nor adrenaline levels in each sample were corrected for variations in the extraction and analytical procedures using the measurements made of the internal standard DHBA (Moyer & Jiang, 1978).

Results

Brief clinical details of the patients investigated in the present study are given in Table 1. A summery of the results obtained from the biochemical assay of nor adrenaline from the corpus cavernosum and tunica albuginea tissues in 4-groups of patients is given in Table-2. These results clearly revealed that the mean nor adrenaline content of corpus cavernosum from group 1 patients was 0.50 ± 3.4 ug/gm tissue, which was significantly higher ($P<0.02$) than the nor adrenaline content of tunica albuginea (0.27 ± 2.4 ug/gm tissue).

In group II patients who had undergone a cystectomy, nor adrenaline content was found to be highest in the tunica albuginea of any of the patient studied ($P < 0.005$). In contrast to group I patients, all the patients (both IDDM and NIDDM) from group IB & IV with clinical evidence of neuropathy had lowered levels of nor adrenaline in corpus cavernosum than the tunica albuginea ($P< 0.02$), however a non significant association was observed when the values of nor adrenaline content in corpus cavernosum and tunica albuginea were compared among IDDM and NIDDM diabetic neuropathic patients. When the nor adrenaline content of corpus cavernosum was compared in group I, III and IV patients, these values were always found to be significantly low in both IDDM and NIDDM diabetic neuropathic patients, however these values for tunica albuginea were within the range predicted from group I patients.

Discussion

Neuropathy is an important feature of diabetic impotence. Studies of this kind are generally faced with considerable difficulties with regard to the availability of both suitable control material and large patient numbers. In previous studies control penile tissues were obtained from transsexual and carcinoma patients undergoing surgery (Melman et al., 1979; Melman et al, 1980). However on occasions, patients with

Peyronie's disease have been used as controls in combination with undergoing penectomy (Gu et. al., 1984; Melman et al., 1979). In an electron microscopic study abnormalities were observed in the myelinated axons within the fibrous plaque, while the unmyelinated axons of the surrounding erectile tissue appeared unchanged, thus there was no ultra structure to suggest that autonomic innervation of penis was affected in Peyronie's disease.

In the present investigation we have therefore compared five patients of Peyronie's disease with five other patients in whom neuropathy was not present clinically nor was there any bladder dysfunction by considering the fact that penis and bladder share a common nerve supply (Faerman et al., 1974; Leu et al., 1984). This was considered the most appropriate criterion for establishing Group I. The results of Group I patients were therefore considered to form a baseline non-neuropathic values against which other groups could be compared.

Our results reveal the existence of a linear relationship between the nor adrenaline levels of corpus cavernosum and tunica albuginea from Group I patients. The nor adrenaline levels in control penile tissue, reported in a previous study (Mel-man et al., 1980) were very variable, probably due to regional variation in tissue sample obtained from penectomies. While patient to patient variability was less marked in the present study, it was possible to compare the individual results obtained for each Group II, III and Group IV with the entire range of values in Group I, as well as to analyze the differences between the mean values for each group. In the present study, all the diabetic neuropathic patients (Both IDDM & NIDDM) revealed a significant preferential decrease in the nor adrenaline content of the corpus cavernosum relative to the tunica albuginea, however we could not find any significant difference in the content of non adrenaline in corpus cavernosum and tunica albuginea among insulin dependent and non insulin dependent diabetics having an objective evidence of neuropathy which suggests that damage to the sympathetic nerves of the erectile tissue had probably occurred in these patients as a result of neuropathy, which is irrespective of their type of diabetes. A significant reduction in the nor adrenaline level in erectile tissue in diabetes associated with impotence has been reported previously, although levels in the tunica albuginea were not measured (Melman et al., 1980). Unlike the present study, reduction was not found in non-insulin dependent diabetics with clinical symptoms or neuropathy. As the clinical details of the patients in the previous studies were not given, therefore, it can not be excluded that non-insulin dependent diabetics with neuropathy maybe as susceptible to sympathetic nerve dysfunction in the penis as insulin dependent diabetics and our present study confirms this hypothesis.

Table 1
Clinical manifestatons of neuropathy in 4 groups of subjects investigated

Subjects		Clinical Manifestations of Autonomic Neuropathy								Other features
		Age (Mean ± S.D.)	Epigastric Fullness	Postrual Hypo-tension	Nocturnal Diarrhea	Swea-ting	Hypo-gastric Un Awareness	Impo-tence	Neuro-pathy	
Group I	Non-Diabetric Non-Neuropathic N = 10	53 ± 3.4	1/10	0/10	0/10	0/10	0/10	10/10	–	Peyronie's Disease: 5/10 Psychogenic Impotence: 5/10
Group II	Non-Diabetic Neuropathics N = 5	50 ± 3.2	4/5	2/5	1/5	2/5	0/5	5/5	+	Cystectomy/Bladder Dysfunction: 5/5
Group III	IDDM-Neuro-pathics N = 10	52 ± 5.4	9/10	9/10	8/10	8/10	9/10	10/10	+	IDDN Diabetes Mellitus/Neuropathy: 10/10
Group IV	NIDDN-Neuro-pathics N = 10	52 ± 4.4	8/10	9/10	8/10	9/10	9/10	10/10	+	NIDDM Diabetes Mellitus/Neuropathy: 10/10

N = Total number of subjects investigated
IDDM = Insulin dependent diabetes mellitus
NIDDM = Noninsulin dependent diabetes mellitus

Table 2
Comparative studies of nor adrenaline content of corpus cavernosum and tunica albuginea in 4 groups of subjects investigated

	Subjects	Nor Adrenaline content (ug/gm) (Mean ± S.D.) CC	TA
Group I	Non-Diabetic Non-Neuropathics N = 10	0.50 ± 3.4	0.27 ± 2.4
Group II	Non-Diabetic Neuropathics N = 5	0.30 ± 2.5	1.35 ± 2.8
Group III	IDDM Neuropathics N = 10	0.20 ± 1.1	0.27 ± 1.9
Group IV	NIDDM Neuropathics N = 10	0.19 ± 1.2	0.28 ± 1.6

N = Total number of subjects investigated
CC = Corpus cavernosum
TA = Tunica albuginea
IDDM = Insulin dependent diabetes mellitus
NIDDM = Non insulin dependent diabetes mellitus

The present study provides no information on the autonomic innervation of the penis in normal potent males, due to the obvious lack of available tissue, although certain patients (with Peyronie's disease and psychogenic impotence) retained some erectile function. The aim of present study was to investigate whether different causes of impotence could be distinguished by the changes in the pattern of autonomic innervation that the erectile tissue exhibited. The results reported here indicate that this was still possible despite the lack of unequivocal control material. The present study does not exclude the possibility that the vascular changes also contribute to the development of impotence. Furthermore, psychological factors in impotence are as important in diabetic as in non diabetic population. Vascular occlusion has been indicated as the major contributing factor by some workers (Herman et al., 1978; Lehman & Jacobs, 1982), while others have found a great association with neuropathy (Karacan, 1980). The results of the present study thus provide evidence that an under-lying neuropathic mechanism itself causes vascular as well as metabolic changes in the adrenergic nerves of the penis in both IDDM and NIDDM diabetic men with neuropathy in addition to the effect of impotence, while in non neuropathic diabetics disease itself causes vascular changes and thus may contribute to the development of impotence irrespective of their type of diabetes.

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